PUBLIC HEALTH LABORATORY WORK

(CHEMISTRY)

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PREFACE

THIS book deals almost exclusively with the Chemical branch of Public Health Laboratory work. The growth of the range and importance of Bacteriology in the work of the Public Health Laboratory have rendered it no longer possible to deal with both subjects satisfactorily within the limits of one handy volume. It is felt, therefore, that the time has now come to exclude all but occasional references to bacteriological matters, and to provide a companion volume dealing with Microbiology from the same practical public health standpoint. This is being prepared by Dr. Sheridan Delépine.

This volume does not describe a large number of methods to the same end. It has always been the aim of the writer to select those processes which experience has proved to be most suitable to the needs of the public health worker.

In the section on Food, while prominence is given to adulteration which raises the presumption of a danger to health, it has not been judged wise to exclude all reference to other forms of sophistication; for the public health student is still required at some examination centres to show a knowledge of these.

I have to acknowledge gratefully my indebtedness to Mr. F. Marchant for assistance rendered in the preparation of this new edition, and I have also to thank Messrs. Townson and Mercer, Messrs. Baird and Tatlock, and others, for the loan of blocks.

H. R. K.

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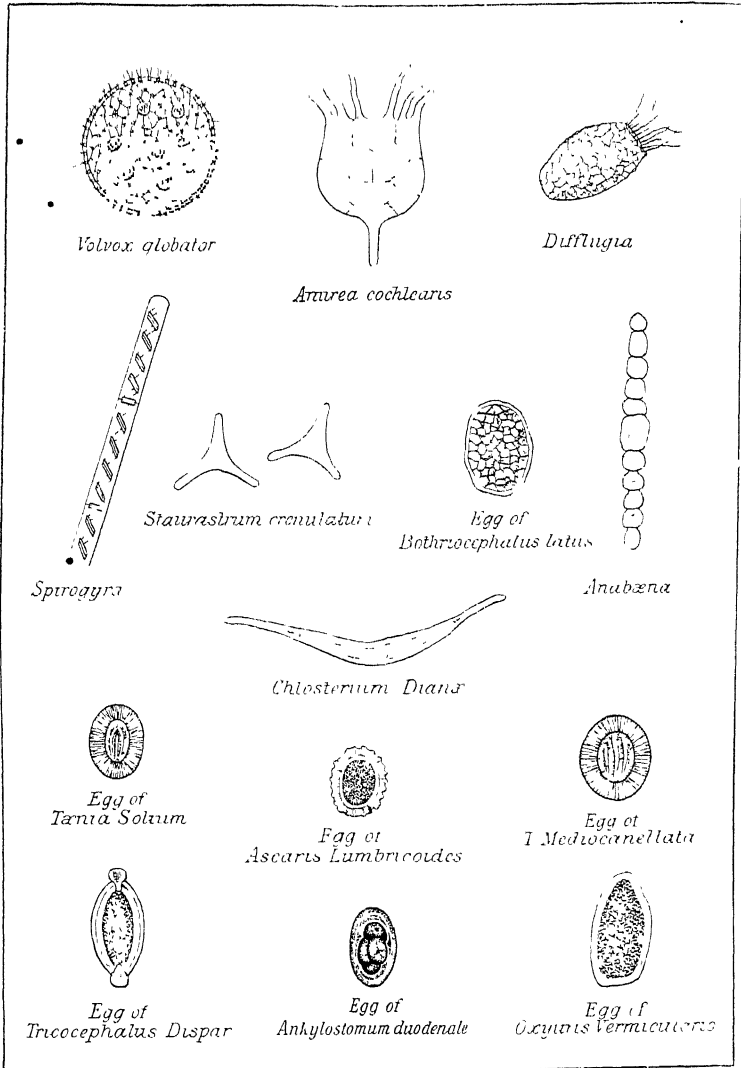
OBJECTS FOUND IN IMPURE WATER (VARIOUSLY MAGNIFIED)

PUBLIC HEALTH LABORATORY WORK

INTRODUCTORY NOTES

The Collection and Weighing of a Precipitate.

The substance is precipitated from a known bulk of liquid, and the precipitate is collected on a filter-paper which has been folded and placed inside a glass funnel. The filter-paper should never project beyond the funnel, and the fluid should be conducted by a glass rod on to the filter-paper, to prevent loss.

Special filter-papers are sold which yield an ash which is generally quite insignificant; and the amount of ash furnished by them is a definite and known quantity for each paper. Such papers should be always employed for collecting precipitates which have subsequently to be ignited and weighed.

The precipitate on the filter-paper is next washed with distilled water from a wash-bottle. A fine jet of distilled water, either hot or cold, is generally employed for this purpose. The process of washing is complete if a drop of the last washing yields no residue when evaporated on a platinum spatula.

The precipitate on the filter-paper is then dried in a drying-oven.

Fig. 1 represents Wills' combined water-bath and drying-oven. It consists of a hot-water bath with openings for evaporating dishes, a spacious hot-air chamber, a pair of hot-water funnels for filtering fatty substances which tend to solidify when cool, and a hot-air box for drying test-tubes, etc. The thermometer, *in situ*, registers the temperature in the interior of the oven.

The filter-paper should then be folded up, placed in a small porcelain crucible (previously weighed), and covered by a lid; the filter-paper and precipitate are next ignited to dull redness, at first gently so as to obviate spurting and loss, but the lid should be removed after a little so as to permit free access of air. It is also desirable to keep the porcelain dish on the slant during ignition, since this favours air draught. When the filter-paper has been entirely destroyed, the capsule and its contents are allowed to cool under a desiccator, and weighed. The weight found, minus that of the crucible and the ash of the filter-paper,
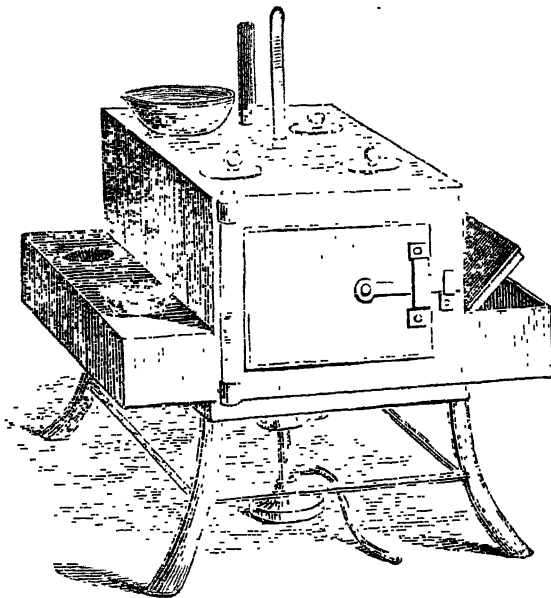
FIG. I.—WILLS' WATER-BATH AND DRYING-OVEN.

represents the weight of mineral precipitate. Care must be taken to remove the dish from the flame *immediately* all evidence of charring or discoloration has disappeared, and not to conduct the incineration at a higher temperature than is found absolutely necessary, or there may be a considerable loss in the mineral residue. Such loss is most generally from ammonia salts (by volatilization), from nitrates and nitrites (by loss of oxygen), from certain chlorides, such as sodium and potassium chlorides (by volatilization), from combined carbonic acid, and from the

water of hydrated salts (such as calcium sulphate), which thereby become anhydrous.

A desiccator is simply a glass shade inside of which there is a vessel containing some agent which will free the air from moisture (such as strong sulphuric acid or solid calcium chloride). A residue completely dried by heat will absorb a little of the vapour from the atmosphere while cooling, and thus increase slightly in weight, unless the precaution is taken to place it inside a desic-
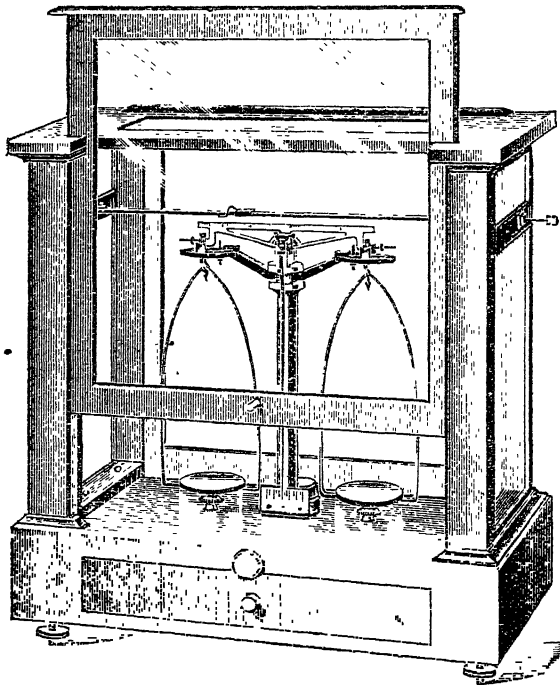
FIG. 2.—CHEMICAL BALANCES.

cator during the cooling process. In the desiccator a perforated tray or a tripod supports the substance to be cooled, and the rim against which the cover closely fits is greased with tallow so that the desiccator is hermetically sealed.

The balances shown in Fig. 2 will be found to be suitable to all weighing purposes. They consist of a short beam which supports two pans, the ends of the beam being constructed with straight knife-edges of agate, upon which the pans are suspended by aagte planes. The case is fitted with a sliding window in

front, which, even when closed, admits of the working of the scales by means of turning a screw which projects externally. The balances must be kept in a dry room, away from any fireplace or door, and placed on a perfectly firm and level surface.

The operation of weighing consists of first lifting the beam off its support by turning the screw, and then noting, by the long indicator which hangs down in front of the central vertical support of the balances, whether the two pans exactly counter-balance each other; if not, the balance must be adjusted by means of a small mechanism situated on the top of the centre of the cross-beam, which can be moved to the right or left, according as it is necessary to increase the weight in either of these directions.

After thus seeing that the scales are accurately equipoised, the material is then placed upon one of the trays, and the weights are added to the other.

After each alteration made in the weights, the result must, of course, be tested; and before any further addition or removal is made the scales must be brought to rest upon their supports, or the apparatus may be put out of gear.

Each of the weights is marked. The larger brass weights (1 to 50) represent grammes, the next in size decigrammes (0·1 to 0·5), the next centigrammes (0·01 to 0·05); and small forceps are used for picking up and applying them to the pan. The milligrammes are added by a little piece of bent wire (the "rider"), which is carried by means of a sliding-rod moving just above the level of the cross-beam, which beam bears markings numbered from 1 to 10. By sliding the rod, the "rider" may be carried to, and placed upon, any one of these marks, when that number of milligrammes of weight will have been added. Each milligramme division is further subdivided to $\frac{1}{5}$ parts of a milligramme.

Example.—A small platinum dish is placed on the left-hand pan.

A 5-gramme weight is placed on the other pan.

The beam is raised by means of a half-turn of the screw, when the platinum dish is found to be heavier than the 5 grammes.

The scales are put at rest by reversing the screw to its original position, and a 2-gramme weight is added to the 5. This is

also carried up by the greater weight of the platinum dish. Another gramme is added; and, being found to be too much, is removed. The dish therefore weighs between 7 and 8 grammes.

A 5-decigramme weight (*i.e.*, 0·5 gramme) is added. The platinum dish is still slightly the heavier; therefore another decigramme is added, with the result that the weights now slightly overbalance the dish. The 1-decigramme weight is removed. The dish therefore weighs 7·5 grammes, but not 7·6 grammes.

A 5-centigramme weight (0·05) is added. This is not enough; but a 2-centigramme weight further added so extremely nearly establishes the required equilibrium that the addition of another centigramme is found to be too much. Therefore the dish weighs 7·57 grammes, but not 7·58 grammes.

Three milligrammes, added by means of the little "rider," make the long indicator oscillate quite evenly on either side of the central mark on the piece of porcelain, where it would ultimately come to rest.

The weight, therefore, of the platinum dish is:

7 grammes	=	7
5 decigrammes	=	0·5
7 centigrammes	=	0·07
3 milligrammes	=	0·003
Total	=	7·573 grammes.

THE COLLECTION AND WEIGHING OF A SOLID RESIDUE AND MINERAL ASH.

A given weight of the liquid is placed in a clean weighed platinum dish. (A platinum dish is cleansed after use with a little dilute hydrochloric acid; then well washed in pure water; and finally heated to redness in the Bunsen flame. It should be allowed to cool under the desiccator prior to weighing.) The dish and its contents are then placed upon a water-bath.

A water-bath is a receptacle which holds water, and when vessels containing liquids are made to stand over the heated water, evaporation of their fluid contents may be effected at a temperature which can never quite reach that of the boiling-point of water.

The water-bath must not be allowed to boil dry. Fig. 3 shows

an arrangement by which this may be guarded against, by the maintenance of a constant water-level in the bath.

When the contents of the dish have evaporated to dryness, the dish is placed in the desiccator for half an hour to cool. It is then weighed, and the solid residue is the weight obtained less the weight of the platinum dish.

The dish is then held by a pair of crucible tongs (which may, with advantage, be platinum-pointed) in the flame of a Bunsen burner until nothing but the mineral ash remains. Fletchers'
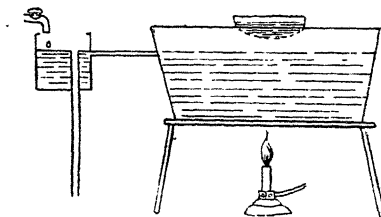
FIG. 3—WATER-BATH WITH CONSTANT WATER-LEVEL.

burners are an improvement upon the common type of Bunsen burner, when it is required to employ a very small flame.

The mineral ash is allowed to cool in the desiccator and then weighed.

Specific Gravity or Relative Density.

The relative density or specific gravity of a solid or liquid is generally referred to water, taken as unity or as a thousand. Where possible, the test must be applied at the temperature of 15·5° C.; but in the case of fats a higher temperature is necessary in order to obtain them in a liquid state.

The student is already familiar with the float instruments or hydrometers which are commonly in use for obtaining specific gravities, and it is only necessary to point out the importance of verifying all these instruments prior to use, by comparing their indications with the results obtained by more delicate methods.

The most accurate estimates of specific gravity are obtained by actual weighings in the specific gravity bottle. The method may be illustrated by indicating how the specific gravity of butter-fat would thus be obtained.

1. A quantity of the butter is heated to, and maintained at, about 65° C. in a water-bath made by standing a small beaker containing the butter in a larger beaker containing water.

2. The fat slowly separates and forms an upper stratum, which rests upon a lower stratum of the water, curd, and salt.

3. In the course of time the upper layer of butter-fat gets clearer and clearer, until at last, all the water, curd, and salt having separated, it becomes clear and transparent. Immediately this has taken place the fat is decanted on to a fine dry filter, in order to guard against the presence of traces of curd and salt; and the filtrate of pure butter-fat is collected and poured into a specific gravity bottle. The specific gravity bottle is a small vessel of thin glass, fitted with a thermometer which also forms a stopper to the bottle and which registers the temperature of the contained liquid, so that this may be known at the moment of weighing. This bottle must be accurately filled and then
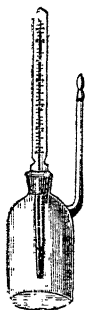
FIG. 4.—A SPECIFIC GRAVITY FLASK.

stoppered, care being taken that no air-bubble or empty space is allowed to remain between the stopper and the liquid.

4. The temperature at which the fat is poured into the specific gravity bottle should be a fraction above 38° C., when the bottle and its contents are transferred to the balance and weighed.

The precise weight must be taken when the thermometer registers exactly 38° C., the flask is entirely filled with the fat, and there is no evidence of air-bubbles.

The weight of the specific gravity bottle when completely filled with distilled water and closely stoppered at the temperature of 38° C., has been previously taken. By a comparison of the respective weights of the two fluids when occupying the flask at the same temperature, the specific gravity of the butter-fat is obtained, that of distilled water being taken as 1,000—

$$i.e., \text{ S.G.} = \frac{\text{The weight of the fat at } 38^\circ \text{ C.}}{\text{The weight of the water at } 38^\circ \text{ C.}} \times 1{,}000.$$

38° C. is here selected as the temperature for weighing because it is the lowest temperature to which it is quite safe to reduce the contents of the bottle without any solidification ensuing, all the fats (animal and vegetable) used as adulterants of butter remaining liquid at that temperature.

The Westphal balance registers the specific gravity on the principle that a body immersed in a liquid loses a part of its weight which is equivalent to the weight of the displaced liquid. The apparatus (Fig. 5) has a swinging arm, which rests on a
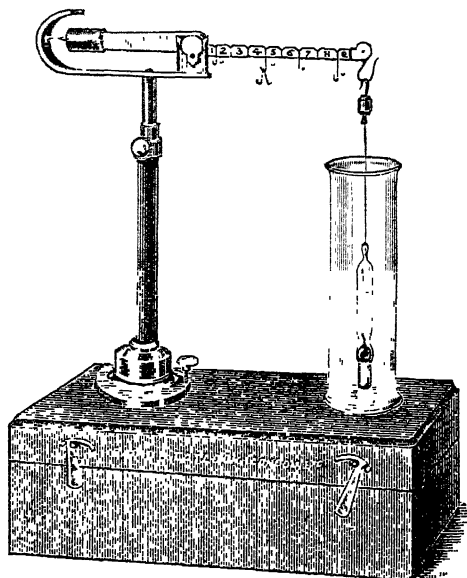
FIG. 5.—THE WESTPHAL BALANCE.

knife-edge, and the upper surface of a part of the arm is notched and graduated. At the free end of the graduated part of the arm is a hook, by which a glass plummet is suspended by means of fine platinum wire. Three different-sized riders (or weights) are provided, of which the largest indicates hundreds, the next tens, and the smallest units. At the other end of the arm is a metal pointer, and the balance prior to use must be so adjusted that, with the plummet immersed in distilled water, and the largest rider placed on the hook (which represents the tenth notch, or 1,000), this pointer rests opposite a small projection on the frame. The adjustment is made by means of the small screw shown on the vertical support of the frame. The liquid

is placed in a glass cylinder and the plummet just completely immersed in the liquid, and by placing the riders on various notches the two pointers are again brought opposite to each other. If, for instance, in order to obtain this result, the largest rider is on the ninth notch, the next largest on the seventh, and the smallest on the fifth, the specific gravity would be 975.

A correction for temperature is necessary for an exact observation by float hydrometers, since all such instruments are originally graduated by water at the temperature of 15·5° C., and the specific gravity varies with the temperature. Within the ordinary ranges of temperature in a laboratory it is sufficient to add 1° of specific gravity for every 3° of temperature above 15·5° C., and to subtract 1° for every 3° below 15·5° C.

The Extraction of Fat by Soxhlet's Apparatus.

Soxhlet's apparatus is shown in Fig. 6. A is the small flask which has been thoroughly dried and weighed and then about half filled with ether; the extraction apparatus is shown attached to the flask between it and the condenser (K). F represents a piece of fat-freed paper containing the substance to be extracted; this is placed in D, care being taken that it is entirely below the level of the small siphon E, so that it may be completely immersed in the solvent, and also that it does not close the opening to the siphon.

The weighed flask of the Soxhlet should have a capacity of about 150 c.c., and contain about 75 c.c. of ether.

The dish on which the flask stands is partially filled with water, and this is cautiously heated; the ether vapour then ascends G, passes into the condenser, and is at once condensed and drops on to F; the ether goes on accumulating, rising the while in the ascending arm of E, until it reaches the level of the upper bend and overflows, when siphonage takes place, and the ether passes out of D back to the flask. Thus the circulation of the ether is completed every few minutes. Immediately after a siphon discharge has returned all the ether to the flask, the latter is removed, the ether driven off over the water-bath at a temperature sufficient to make the ether boil, after which the flask and its contents are dried at 100° C. until a constant weight is obtained.

Of course, there must be no doubt as to whether the extrac-

tion has been complete; this may be tested by fixing a second small flask containing more ether, and after about half an hour evaporating off the ether and drying at 100° C.; it can then be noted whether there is any material increase over the original weight of the flask.

It is well to place a small plug of blotting-paper in the mouth of the open tube at the top of the condenser so as to limit the
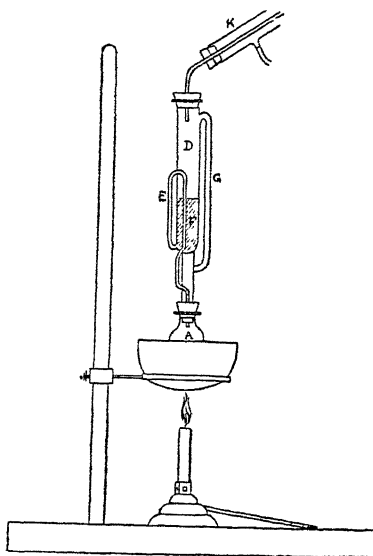
FIG. 6.—SOXHLET'S FAT-EXTRACTION APPARATUS.

access of air, the moisture of which would otherwise condense and slightly wet the ether.

THE SPECTROSCOPE.

A knowledge of the spectroscope is useful to the public health worker, and for those unacquainted with the use of this instrument a brief description is given.

If a compound light, such as sunlight, is made to pass through a glass prism, the different coloured rays of which it consists are unequally refracted (or bent out of their original course), so that beyond the prism they form, upon a white surface, a continuous line of colours called the "spectrum"; and the spectrum of the compound white light will be seen to consist, in order from right

to left, of red, orange, yellow, green, blue, indigo, and violet. A number of dark lines—called "absorption bands" or "Fraunhofer's lines"—are also seen to cross the image of the solar spectrum. These lines indicate the absence of rays of certain refrangibilities from the beam of solar light; each occupies a definite position, and therefore affords a means of accurately localizing the parts of the spectrum.

In other lights the spectrum will only show a few *bright* bands (that of the sodium flame only *one*), and the remainder of the spectral image is almost—or quite—invisible by comparison.

If we transmit solar light through different coloured solutions, we then get different absorption bands. If a solution of fresh blood, for instance, be taken, and a small colourless cell containing it is placed before the slit in the instrument which admits the
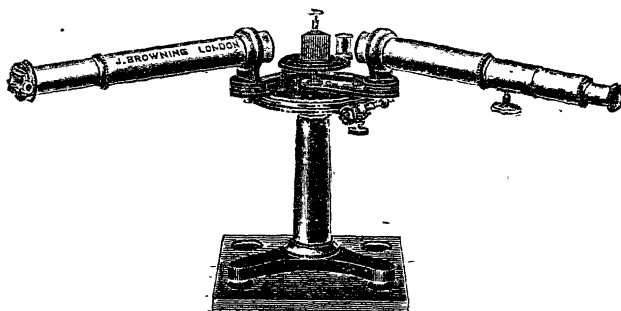
FIG. 7.—THE SPECTROSCOPE.

light, two distinct and characteristic dark stripes or absorption bands appear in the yellow and green parts of the solar spectrum.

Fig. 7 will serve to show the manner in which a spectroscope is constructed.

A firm iron stand is seen to support at its upper end a brass plate carrying the glass prism; laterally, a cylinder is also fastened to the brass plate, and in the end of this cylinder which is nearest the prism a lens is placed, the other end being closed by a plate with a vertical slit in it (the width of which can be regulated by a screw to meet requirements); through this slit the light is admitted to the prism, the rays first passing through the lens and thereby being rendered parallel and condensed. The spectroscopic appearance is then viewed through a small telescope (with a low magnifying power), and this (the tube on the

right as seen in the figure) is fitted on to the cast-iron foot so as to be movable in a horizontal plane about the axis of the foot. The telescope is made to move over a scale which can be read with a vernier.

All foreign light must, of course, be cut off; and this may be done by a black cloth, which is thrown over the prism and the tubes.

The slit may be furnished with a reflecting prism, by means of which two spectra can be compared at the same time.

Thus, by a spectroscopic examination, the colour, number, and position of the bright lines on the spectroscopic scale may be carefully observed and noted. If it is desired to distinguish metals by means of their spectral lines, the substance is dissolved in a drop of the purest hydrochloric acid; a piece of recently ignited platinum wire is then dipped in the solution and held in a Bunsen flame.

A convenient method of performing spectroscopic observations is by means of the Sorby-Browning micro-spectroscope, which consists of a small spectroscope placed in connection with a microscope in such a way that the former fits into the tube of the latter, similar to an eyepiece.

THE POLARISCOPE.

A simple form of half-shadow polariscope consists of a horizontal brass tube mounted on a vertical stand, and having a Nicol prism at each end, one being the "polarizer," and the other the "analyzer." A monochromatic light, such as the yellow sodium flame (which may be obtained by placing a platinum cup containing sodium chloride in the flame of a Bunsen burner), is admitted to the polarizer. At the opposite end of the brass tube an eyepiece is fitted just in front of the analyzer. In the brass tube can be placed a clean and dry glass tube containing the solution under examination.

Light consists of vibrations of ether in *all* planes, and its transmission occurs in waves; but the monochromatic light consists of light of a single wave-length. The polarizer allows only the vibrations taking place in one plane to pass, others being intercepted. Now, when the analyzer is placed parallel to the polarizer, all the vibrations pass through the analyzer also, and equal illumination is seen on both sides of a sharply defined vertical middle line when looking through the eyepiece, this point of equal illumination being called the "zero-point." The slightest

rotation of the analyzer will then produce a difference in the illumination of the two sides.

In using the instrument, the zero-point is first obtained, with the glass observing-tube filled with distilled water and placed in position; then if some sugar solution (or other optically active liquid, which has the property of rotating the plane of polarized light) be placed in the glass tube, the rays will no longer pass through the analyzer, and the equal illumination is disturbed. If the analyzer be turned round, it is possible to obtain an equal illumination, or, in other words, to compensate for the optical disturbance of the rotating substance; but the direction and the angle through which it has been turned (as indicated on a dial fitted with a vernier) vary with the amount and nature of the rotating substance examined, the number of degrees being termed
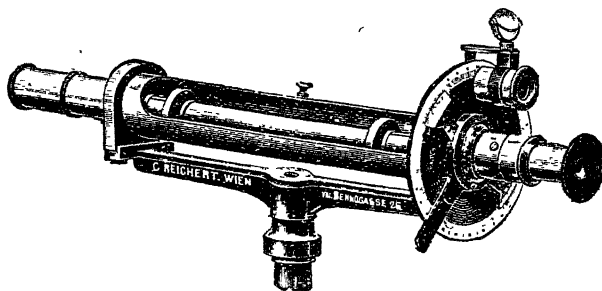
FIG. 8.—THE POLARISCOPE.

the "index of refraction," from which the so-called "specific rotary power" of the substance may be calculated.

The polariscope is used to find the percentage adulteration of butter with other fats (refractometer), and also the strength of saccharine solutions (saccharimeter). With pure butter an equally distributed light can be obtained, but with butter containing fat which has been melted (margarine) this is impossible, since such fats rotate the plane of polarization. Glucose in honey and added sugar to milk may also be detected by this instrument, for while most sugars have the property of deflecting the ray of polarized light to the right (dextro-rotary, indicated by the sign +), others deflect to the left (levo-rotary, indicated by the sign −), and this affords a means of distinguishing between them. If the nature of the substance is known, one can, moreover, estimate its quantity, since 1 gramme of a particular optically active substance has its own specific rotary power.

But for the work demanded of the public health worker it is not necessary to determine specific rotary powers, useful as these may be in some of the work which a public analyst may be called upon to perform. Indeed, from the standpoint of the public health worker the micro-polariscope (in which the polariscope is adjusted to an ordinary microscope) will generally suffice. In this instrument one of the Nicol prisms (the analyzer) is inserted in the brass tube of the microscope immediately above the objective, and the other (the polarizer) is fitted beneath the stage of the microscope, so that the specimen examined on the slide stage of the microscope is now between two Nicol prisms, the lower one of which is the polarizer. Such an instrument will be found of assistance in distinguishing between certain starches, some of which polarize better than others, and in distinguishing between pure butter and margarine. With pure butter a completely dark field cannot be obtained, whereas with margarine from fat which has been melted it can; and in the case of mixtures it is impossible to *completely* obscure the field by rotating the analyzer.

Graduated Burettes.

In working with delicate standard solutions it is best to employ a mounted burette fitted with a stopcock at the bottom, rather than an unmounted one controlled by the finger, as in the former case the possibilities of contamination are reduced, and there is no risk of any loss from the burette while the operator is mixing or colour-matching in the intervals of the addition of further quantities of the standard solution. When a hand-burette is employed the index-finger which controls its delivery must be quite dry.

Unmounted burettes should not be blown out, but allowed to drain, and the drop at the delivery end removed by touching the side of the vessel into which the contents are emptied.

In judging the height to which fluid stands in a burette, always take the level of the concave lower border of the meniscus which forms upon its upper surface, and make this rest upon the line to which the fluid is required to reach. Water standing to the level of 10 c.c. in a burette will appear, therefore, as in Fig. 9. The eye must always be on a level with the upper surface of the liquid when a reading is made.

The burette just holds 10 c.c. of water if at a temperature of about 15° C. the water weighs 9·99 grammes. Similarly, with a 100 c.c. measuring flask the graduation is correct if the 100 c.c. of water, at about 15° C., weigh 99·9 grammes.
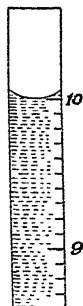
FIG. 9.—A BURETTE FILLED UP TO THE 10 C.C. MARK.

For cleaning glass burettes, etc., and porcelain apparatus especially from fatty matter, the commercial trisodium phosphate is useful.

INTERNATIONAL ATOMIC WEIGHTS (1917). O=16.

Element	Symbol		Weight
Aluminium	(Al)	=	27·10
Arsenic	(As)	=	74·96
Barium	(Ba)	=	137·37
Boron	(B)	=	11·00
Bromine	(Br)	=	79·92
Calcium	(Ca)	=	40·07
Carbon	(C)	=	12·00
Chlorine	(Cl)	=	35·46
Chromium	(Cr)	=	52·00
Copper	(Cu)	=	63·57
Fluorine	(Fl)	=	19·00
Hydrogen	(H)	=	1·008
Iodine	(I)	=	126·92
Iron	(Fe)	=	55·84
Lead	(Pb)	=	207·20
Magnesium	(Mg)	=	24·32
Manganese	(Mn)	=	54·93
Mercury	(Hg)	=	200·60
Nitrogen	(N)	=	14·01
Oxygen	(O)	=	16·00
Phosphorus	(P)	=	31·04
Potassium	(K)	=	39·10
Silicon	(Si)	=	28·30
Silver	(Ag)	=	107·88
Sodium	(Na)	=	23·00
Sulphur	(S)	=	32·06
Tin	(Sn)	=	118·70
Zinc	(Zn)	=	65·37

Weights and Measures upon the Metrical System.

The metrical system is founded upon the "metre," which is divided or multiplied by ten to represent different measures, as follows:

Length.

1 millimetre	=	$\frac{1}{1000}$ part of a metre.
1 centimetre	=	$\frac{1}{100}$ part of a metre.
1 decimetre	=	$\frac{1}{10}$ part of a metre.
1 metre	=	39·37 inches.
1 decametre	=	10 metres.
1 hectometre	=	100 metres.
1 kilometre*	=	1,000 metres.

Capacity.

1 cubic centimetre	=	0·061 cubic inch.
28·35 cubic centimetres	=	1 fluid ounce.
1,000 cubic centimetres or 1 cubic decimetre	=	1 litre.
1 litre = 35·3 ounces	=	1·76 pints.
1 pint	=	568 cubic centimetres = 0·568 litre.
1,000 litres	=	1 cubic metre.
28·328 litres	=	1 cubic foot.

One c.c. of distilled water at 4° C., and 760 millimetres barometric pressure, weighs 1 *gramme*, which is the standard of weight.

Weight.

1 milligramme	=	$\frac{1}{1000}$ part of a gramme.
1 centigramme	=	$\frac{1}{100}$ part of a gramme.
1 decigramme	=	$\frac{1}{10}$ part of a gramme.
1 gramme	=	15·432 grains.
1 decagramme	=	10 grammes.
1 hectogramme	=	100 grammes.
1 kilogramme	=	1,000 grammes.
1 ounce	=	28·35 grammes = 437·5 grains.
1 pound (16 ounces)	=	453·6 grammes = 7,000 grains.
1 gallon of water	=	4·536 litres = 10 pounds.

1 litre of hydrogen at 0° C., and 760 millimetres pressure, weighs 0·0896 gramme.

1 litre of oxygen at 0° C., and 760 millimetres pressure, weighs 0·0896 × 16 grammes.

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* The Latin prefix therefore indicates division, the Greek multiplication.

Thermometer Scales.

Centigrade.	Freezing-point	= 0	..	Boiling-point = 100
Réaumur	,, ,,	= 0	..	,, ,, = 80
Fahrenheit	,, ,,	= 32	..	,, ,, = 212

$$\therefore \frac{Centigrade}{5} = \frac{Réaumur}{4} = \frac{Fahrenheit - 32}{9}.$$

∴ To convert Centigrade to Fahrenheit, × 9 ÷ 5, and add 32.
,, ,, Fahrenheit to Centigrade, subtract 32, ÷ 9 × 5.
,, ,, Réaumur to Fahrenheit, ÷ 4 × 9, and add 32.
,, ,, grains to grammes, × 0·0648.
,, ,, cubic feet to cubic metres, × 0·0283.
,, ,, cubic feet to litres, × 28·3.

PART I

THE CHEMICAL, MICROSCOPICAL, AND PHYSICAL EXAMINATION OF WATER FOR PUBLIC HEALTH PURPOSES

CHAPTER I

THE COLLECTION OF SAMPLES—INFORMATION REQUIRED AS TO SAMPLES—QUANTITATIVE EXPRESSIONS

THE sample should always be collected for analysis just as it is ordinarily obtained for drinking purposes. It is obvious, since our object is to discover all the possibilities of danger, that an endeavour should be made to ascertain the maximum amount of pollution to which the water is liable. For instance, in the case of streams, lakes, etc., the point of entrance of any drains should only be avoided to the same extent as it is by those who may come to collect their drinking-water.

When there is a general system of water-supply, an effort must be made to meet the same ends by choosing samples from the street fountains and street mains, rather than from storage, etc., reservoirs. But since impurities may gain access during domestic storage and distribution, it would not be fair in all cases to judge a public supply from the tap-water of any particular dwelling.

With regard to shallow wells from which the water is removed by pumping, it is advisable to continue the process for some time, but no longer than it is judged that the water may be pumped during any one day, under the prevailing circumstances of demand. This is done because the last "pumpings" will often furnish the maximum evidence of any pollution present.

To ascertain whether the water may have been contaminated during its domestic storage and distribution, the sample should be taken from the lowest draw-off tap (generally the scullery sink tap), as then the water will have run the maximum risk of contamination.

When the fact is borne in mind that the water from many

shallow wells is materially influenced both as to quantity and quality by the rainfall, it will be understood how samples from the same well may vary in purity according as to whether a long dry period may have preceded the collection, or a heavy rainfall, which may be the means of conveying to the well water impregnated with surface washings, or water which may have washed accumulated impurities out of the interstices of the soil. These facts as to rainfall should always be ascertained; and it may be desirable to examine a further sample after prolonged and heavy rain.

The fact as to whether a cesspool or drain contaminates a well can readily be decided either by introducing a considerable quantity of sodium chloride, followed by plenty of water, and estimating the chlorine in the well-water every morning and evening for several days; or by introducing a strongly alkaline solution of fluorescine, and endeavouring to detect the green colour in the well-water.

Water is customarily collected for analysis in a large glass-stoppered bottle, called a "Winchester quart," which holds about twice the amount which is implied by its description (*i.e.*, about half a gallon). Stout wicker covers are made to protect them in transit by parcel post or rail. These bottles have become generally adopted because, in addition to holding an amount which meets all the requirements of an ordinary analysis (even though it be necessary to repeat some of the estimations), they are strongly made; but obviously any stout glass bottle of similar dimensions, fitted with a glass stopper, will serve the same end. If a mineral analysis should be required, it is necessary to have quite 2 gallons of the water. It is well to avoid the employment of stoneware bottles.

The bottle must be thoroughly cleansed by first well rinsing with a little dilute hydrochloric acid, and then by washing in good water until the washings are no longer acid.

In collecting a sample the bottle is first quite filled with the water, and then emptied; it is again almost completely filled up (in a manner which will not favour the aeration of the water), and the glass stopper, having been found to fit accurately and tightly, is well rinsed in the water before it is inserted, when it is tied down firmly on to the neck of the bottle and the knots are protected with sealing-wax. In collecting a sample from a stream the bottle should be grasped near the bottom, held well

under the surface of the water, with its mouth pointed up-stream, so that the water does not flow over the hand into the bottle. Care is taken to keep the sample cool and unexposed to light until the analysis is commenced; and under no circumstances should some of the estimations be unnecessarily delayed, as important chemical changes may occur—*i.e.*, organic matter may suffer a very slight reduction, free ammonia may increase or decrease in amount, nitrates may be reduced or even increased, calcium or magnesium carbonates and iron, which were held in solution by carbonic acid, may, owing to the escape of the carbonic acid, be partially deposited. Therefore the figures of the two ammonias, the oxidizable organic matter, and of the oxidized nitrogen, together with the physical characters, should always be ascertained as soon as possible (and certainly within forty-eight hours) after the sample has been collected.

Information required as to Samples.

It is often difficult to form a correct opinion upon the purity of a sample without the knowledge of some of the circumstances of its source; and if the water is held to run risk of harmful pollution this should suffice for its condemnation, although the chemical analysis at the time may prove satisfactory; natural agencies may suffice to purify water for a time, but there is always a possibility of their purifying powers being exhausted at any moment, and the danger of drinking such water is a constant one.

Thus, information as regards the risks of pollution may be of great value as indicating possibilities of danger, when such danger may not be manifest at the time by analysis; it is also of value to ask, in every instance, the motive for requiring an analysis.

Information bearing upon the constitution of the strata through or over which the water has passed is most valuable, since the soluble mineral constituents of certain strata are similar to those which may result from previous organic pollution. Anyone is able to furnish information as to whether the surface consists of such familiar substances as clay, gravel, sand, chalk, or vegetable mould, and whether the subsoil, exposed as it is by railway or road cuttings, is of chalk, sandstone, etc.

It is very desirable that labels should be given to those collecting samples, and that these should be affixed to the bottle. The subjoined label, when filled in, would convey all necessary information to the analyst:

SAMPLE OF WATER FOR ANALYSIS.

Name and address of sender ....
Place, date, and hour of collection ....
Source of sample and method of collection ....
If from well, give approximate depth............ ....
and geological characters of the soil and subsoil of the district
....
If from shallow well, give the rainfall during the previous week, in such terms as " nil," " small," or " great " in amount ....
Nature and distance of any evident or possible source of pollution
....
Reason for desiring an analysis ....

The following is the usual form of report upon the chemical examination of a sample of water:

Report on the Analysis of a Sample of Water received on ....
from ....*and labelled*..................
....

Number of sample .. ..
Date of examination .. ..
Physical characters .. ..

Reaction .. ..

	Parts per 100,000.
Saline and free ammonia	
Organic (or " albuminoid ") ammonia	
Oxygen absorbed from permanganate in two hours at 27° C. ..	
Chlorine..	
Nitrogen as nitrates	
Total solid matter	
(*a*) Volatile	
(*b*) Fixed	
Appearance on ignition	
Total hardness	
(*a*) Temporary	
(*b*) Permanent	
Poisonous metals	

Nitrites .. ..
Phosphates .. ..
Sulphates .. ..
Microscopical examination of the sediment.. .. .. ..

Opinion...
....
....
....

(*Signed*) ....

Date........................

Where a series of analyses are to be brought into comparison, the following form of report is to be preferred:

RESULTS OF ANALYSIS EXPRESSED IN PARTS PER 100,000.

Numbers of Samples.	Description of Samples.	Saline and Free Ammonia.	Organic or Albuminoid Ammonia.	Oxygen absorbed in Two Hours at 27° C.	Chlorine.	Nitrogen as Nitrates and Nitrites.	Hardness.			Solids.			Remarks.
							Temporary.	Permanent.	Total.	Volatile.	Fixed.	Total.	

The result of every analysis should be carefully entered in a book kept for the purpose, for such a record becomes most valuable for reference purposes and for making comparisons with future samples of water from the same locality.

The results of the estimations made in water analysis are still variously returned in terms of grains per gallon and parts per 100,000.

It seems desirable that uniformity of expression should be established. Parts per 100,000 is the most common return made in this country, and it is, moreover, in general use in France, Germany, etc.

It is easy to convert grains per gallon to parts per 100,000, or *vice versa*.

Supposing a report reads "chlorine 2·8 grains per gallon," how many parts per 100,000 will this represent?

There are 70,000 grains in a gallon. Therefore there are 2·8 grains in 70,000 grains, or 2·8 parts per 70,000 parts, or 4 parts per 100,000.

It is only necessary, therefore, to multiply results returned in "grains per gallon" by ten, and to divide by seven, in order to convert them into "parts per 100,000," since grains per gallon are parts per 70,000; and so, to convert "parts per 100,000" to "grains per gallon," the returns must be multiplied by seven and divided by ten.

Where the results of a quantitative test are returned in terms of "grains per gallon," it is convenient to work with 70 c.c. of the sample. The reason for this is that 70 c.c. represent "a miniature gallon," so called, and the results can at once be

expressed in terms of an imperial gallon. The relation between the so-called "miniature gallon" and the imperial gallon depends upon the following facts:

One c.c. of water is taken to weigh 1 gramme. Therefore 70 c.c. ("the miniature gallon") of water weigh 70 grammes, or 70,000 milligrammes.

Therefore, since there are 70,000 component parts in each case, the milligrammes in "the miniature gallon" are equivalent to the grains in the imperial measure.

The various tests employed in the chemical and physical examination of water for public health purposes have now to be considered. But it must be fully realized that organic pollution will give evidence of its presence in many of the steps which form a complete analysis, and that it is this collective evidence which determines the opinion to be formed, and not the evidence which any one special test may appear to offer.

CHAPTER II

THE PHYSICAL CHARACTERS OF WATER

WHEREAS polluted shallow-well waters are notoriously often clear, sparkling, and pleasant to the palate, these characters are precisely those of our purest and best waters. For this reason, and from what follows, it will be seen that the evidence of purity furnished by the senses may be very misleading. Such physical tests, therefore, are not worthy of lengthy consideration.

THE PHYSICAL CHARACTERS.

The sample is first well shaken, and then a thin, colourless glass tube, 24 inches long, is filled with the water, and from the appearance of this, in the "2-foot tube," as it is called, the physical characters of clearness or turbidity, colour, and the degree of aeration, are noted.

1. **Clearness.**—Though the best waters are always bright and clear, these qualities cannot be considered as evidence of purity, for a polluted well-water may also possess them; and, on the other hand, any slight haziness or turbidity—which is, of course, furnished by minute particles of suspended matter—may by chemical and microscopical examination be proved either innocuous or harmful. Opacity may be caused by clay, iron, chalk, lead, or vegetable matter in suspension.

It is difficult to satisfactorily estimate the amount of turbidity. Generally the transparency of water is measured by ascertaining through what depth of liquid a black-and-white figure can be seen with a given intensity of light. The degree of any such turbidity may be expressed as "very slightly turbid," "slightly turbid," and "turbid."

2. **Colour.**—To detect this the tube is fixed vertically so as to stand upon a white porcelain slab, and the observer looks down through the depth of the column of water on to the slab, which

then forms a background. It is only in this matter that the faintest degrees of coloration are best appreciated, if even they be detected at all; and when thus examined it is rare that the water is not seen to possess a colour, however faint. In a good water it is generally of an extremely faint greyish-blue or greenish tint. Filtration of the water would show whether the colour is due to suspended or dissolved matter.

The colour, which is most marked in water from reservoirs, lakes, and rivers, tends to a greenish hue in the spring and a brown in the autumn and winter.

The various hues of yellow and brown will denote either the presence of animal or vegetable pollution (*i.e.*, sewage or peat), or mineral contamination, such as iron or clay; but if such colour is due to iron or clay a sediment will form. Clear waters from a depth which turn to a brownish-yellow colour on standing contain iron, the soluble ferrous salts becoming oxidized to insoluble ferric salts.

The amount of sewage pollution must be enormous to furnish any colour, and a brownish tint in waters used for drinking purposes is generally due to peat or iron.

A brown or red colour associated with turbidity and odour is often due to the growth of *Crenothrix polyspora*, a microscopic vegetable growth consisting of massed zooglœæ and slender cobweb-like filaments. The organisms form a great number and diversity of spores, and hence its specific name of polyspora. The growth is rich in iron.

A marked green denotes the presence of vegetable matter containing chlorophyll, which will generally be found to consist of the harmless algæ.

A blue-green tint in water, associated with a floating bright green scum, has been found to be due to a species of *Anabæna* in association with a monad (Garrett).

Red rain—in which the colour was produced by dust (probably of cosmic origin)—fell in some parts of Europe in 1901. Rain-water has also been coloured by volcanic ash and by desert sand; and "bloody" snow, produced by growths of *Palmella sanguinea*, has been described.

A red colour has been found to be due to an alga named *Oscillatoria rubescens*.

Colour alone affords no justification for condemning a water as unfit for drinking purposes until the nature of the material

furnishing it is known; peat, for example, present to quite a harmless extent, will often colour a water markedly.

The importance of the test does not seem to warrant any attempt at definite measurement when isolated examples are examined; but this is of service as a rough indication of the working efficiency of filtration processes in the case of some waters. If such a test is desired, it is best performed by the method of Crookes, Odling, and Tidy. In this method an empty tube, exactly similar in every respect to one containing the water to be compared, is employed; this has two hollow glass wedges behind it, the one filled with ½ per cent. sulphate of copper solution, and the other with a mixture of ferric chloride (0·7 gramme per litre) and cobalt chloride (0·3 gramme per litre), with a very slight excess of hydrochloric acid. These wedges are made to slide across one another in front of a circular aperture in a metal sheet; and thus any desired combination of brown and blue can be obtained. They are pushed over the empty tube until the colour, on looking down it, appears to be identical with that of the water-tube. Each prism is graduated from 1 to 50, the figures indicating millimetres in depth of the solution at that particular part of the prism, and the degree of colour is expressed as equivalent to so many millimetres of blue and so many of brown solution.

3. **Taste.**—The pleasant taste of good water is furnished by the gases dissolved in it; but since water must contain large quantities of any ingredient for its presence to be detected by the sense of taste, as an indication of dangerous contamination the test is useless. One-quarter grain to the gallon of iron will impart a faint chalybeate flavour, and this amount should not be exceeded in a drinking-water. Chloride of sodium (common salt) may be present in enormous quantities (80 to 90 parts per 100,000) without causing a brackish taste; and waters foully polluted with organic matters are often so palatable that they have frequently been preferred by the public to much purer waters.

Certain vegetable growths (*Anabæna* and *Tabellaria*) may be the cause of unpleasant tastes.

It is not advisable, as a rule, to taste samples sent for analysis.

4. **Odour.**—This is best detected by nearly filling a 200 c.c. glass-stoppered bottle (itself colourless), almost completely immersing this in hot water at about 60° C. for a few minutes, and

then noting any odour immediately on removing the stopper. The preliminary addition to the water of a little strong potassic hydrate solution helps to make the test more sensitive. For most practical purposes, however, it will suffice to smell the sample after it has been thoroughly shaken; and it is only necessary to resort to the plan of heating the water when a suspicion remains after this procedure, or when there is strong reason for considering that odoriferous gases may be present in small quantities inappreciable except when disengaged by heat.

The test of odour is unreliable, and none may be evident in waters which are grossly polluted by sewage; it must be borne in mind, moreover, that many of the noxious materials which may gain access to a water have little, if any, odour originally.

The variety of odours which may be given off from a water defies description; many of them, though quite peculiar and distinct, it is impossible to describe, and any comparisons made with other odours are not always appreciated; but under any circumstances the analyst should attempt to describe the odour in his own words.

Many odours are due to the growth of minute organisms, more especially algæ. *Anabæna* and *Tabellaria* may give rise to unpleasant odours, also *Cryptomonas* and other protozoa. To favour a large growth of algæ the water must be stagnant and quiescent (as in ponds and reservoirs) in situations sheltered from the wind.

Such terms as "musty," "horse-pond-like," "pig-odour," "fishy," "cucumber-like," "grassy," "earthy," etc., have been employed to denote the odour in certain pond waters. Some of these odours are related to essential oils, and are produced by living organisms; others result from the decay of the bodies of these organisms.

A distinctly putrid odour is characteristic of large quantities of decomposing animal or vegetable matter, and a *urinous* odour is sometimes perceptible when large quantities of fresh sewage have gained access to water. The rotten-egg odour of sulphuretted hydrogen and that of coal-gas are both peculiar and distinctive. The presence of any of these odours would condemn the water, except in the case of waters from a depth naturally charged with sulphuretted hydrogen and free from animal pollution (as at Harrogate, etc.).

The fishy odouris generally due to infusorians (*Uroglena* and

other protozoa, *Volvox*, etc.), but it may arise from decomposing algæ. Apparently it may also be due to small water-snails; and the introduction of trout to reservoirs thus affected has, by keeping down their numbers, led to a cure. *Crenothrix*, a fungoid plant which grows in the presence of protosalts of iron and decomposing organic matter, has often given rise (like *Beggiatoa*) to disagreeable odour (SH_2) and taste in public water-supplies, especially in the late summer and autumn. Small water-eels in water-pipes have, by their decomposition, occasioned evil odours in waters.

Sulphuretted hydrogen may mask other odours; the addition of a little copper sulphate solution will prevent this and produce a brownish discoloration (due to CuS).

As a rule the most frequent and objectionable odours are developed in surface waters.

5. **Aeration.**—Evidence of this is afforded by the amount of lustre the water possesses. Coarser degrees of aeration are noted by minute air-bubbles collecting at the sides and bottom of the 2-foot tube, and rising up occasionally through the water to the surface. Waters from the chalk and limestone formations contain much carbonic acid, and little of any other gas; and where the water from these strata has been subjected to conditions of high pressure and low temperature, carbonic acid may even be in such quantities as to give a white turbidity to the water when this is first exposed to the air.

The degree of aeration is of no value by itself in the estimation of the purity or impurity of a water, though a good water, to be palatable, must be well aerated. Many deep-well and spring waters, of great purity, are poorly aerated, and many foul waters are particularly bright and sparkling (chiefly from carbonic acid derived from organic decomposition).

6. **Reaction.**—This is important, not so much as affecting an opinion upon the wholesomeness of the water as from the circumstance that it determines the effect which the water may have upon lead, iron, and zinc surfaces, and because in some steps of the analysis it becomes necessary to neutralize any acidity in the sample. Most waters markedly polluted with animal matter are decidedly alkaline from the carbonate of ammonia furnished by urine decomposition. The majority of pure waters in this country are faintly alkaline, the alkalinity being most generally given by calcium carbonate, and less often by sodium carbonate, etc.

Some waters will be found to be neutral in reaction, and acid waters are by no means uncommon in this country; the latter most frequently obtain their acidity from the peaty acids (the humic, ulmic, and geic) taken up from the decaying vegetable matter encountered in their surface flow, and such acidity is a characteristic feature of so-called "peaty" waters. It is most marked after long periods of dry weather.

The reaction may be obtained by partially immersing in the water pieces of delicate blue and red litmus-papers, and noting the change after a few minutes.

If the water gives an acid reaction, it should be boiled, allowed to cool, and tested again. If the acidity has been lost, it was due to free carbonic acid. Any free acid in water is most generally carbonic acid or organic acids, but it may also be sulphuric acid. The sulphuric acid may gain access to water from the oxidation of iron pyrites (sulphides) in the soil, or, like other acids, from the waste of factories; or in small amount from the sulphur in the coal burnt when rain-water washes the atmosphere over a town. Alkalinity that disappears on boiling is due to free ammonia.

It is not generally necessary for hygienic purposes to test the *degree* of alkalinity or acidity of the water, but it is sometimes useful to make such an estimation, and the method is set forth in Chapter XVI.

7. **The Sediment.**—The presence of this, together with its macroscopic appearance, should be noted at this stage, but any opinion of its nature must be reserved until a microscopic and chemical examination have been made (*vide* Chapter VIII.). Certain waters deposit calcium carbonate or iron when allowed to stand exposed to the air; this is due to the escape of the carbonic acid which held these substances in solution.

8. **Temperature.**—Sometimes spring-water from a great depth is warm or even hot. This is due to the fact that, below the level at which variations due to atmospheric alternations of temperature cease to be recognizable, the temperature of the earth increases with the depth when the measurement commences a few feet from the surface, the water temperature rising about 1° F. for every 50 to 60 feet of depth, on an average. The temperature of water may furnish a useful indication of its source as well as the depth from which it issues.

CHAPTER III

CHLORINE

CHLORINE exists in most waters as chloride of sodium, potassium, or calcium. Rarely free chlorine has been found in waters polluted by industrial waste products.

This combined chlorine is present in all waters to a small extent, even in rain-water. In the rain-water of country districts the chlorine varies from 0·2 to 0·5 part per 100,000.

The presence of combined chlorine in water in excess of this amount is due to one of the following causes:

(*a*) The water has previously percolated strata which yield chlorides, such as greensand, sandstone, the London clay, chalk, etc. In the districts of salt deposits and in certain districts near the coast the well-water may contain very large quantities of chlorides.

(*b*) Pollution by animal organic matter, and chiefly urine (which contains nearly 1 per cent. of chlorides).

(*c*) Admixture with sea-water, as in tidal rivers and occasionally deep wells by the sea-coast.

(*d*) Open reservoirs and other expanses of fresh-water stored near the coast take up chlorides in appreciable amount from the atmosphere. It may also happen that large collections of rain-water show a chlorine figure considerably above that in the rainfall of the district, even when they are not situated near the coast. This circumstance is accounted for by the concentration which the water is always undergoing on account of the evaporation from its surface.

(*e*) Effluents from alkali and other industrial works.

Thus where it is possible to exclude sources (*a*), (*c*), and (*e*), the presence of chlorine in excess of 1 part per 100,000 may be taken

to indicate organic contamination (past or present), and that of an animal nature, since vegetable pollution *per se* furnishes no such excess; hence chlorine often serves to indicate whether a vitiated water is polluted by animal or by vegetable matter.

It remains now to be seen, apart from other information which is available, what *chemical* means there are of excluding causes (*a*), (*c*), and (*e*) as furnishing chlorine in excess of the amount in pure rain and surface waters. When the excess is derived solely from the strata, there will be no evidence of organic pollution furnished by the other steps of the analysis. If, on the other hand, the excess be due to animal pollution, there will be further evidence of this contamination in all those steps of the analysis which serve to indicate such pollution. The amount of chlorine originally taken up from animal pollution is not reduced by subsequent filtration of the water through subsoil or strata.

Finally, if the excess of chlorine be due to admixture with sea-water, there will also be present large quantities of magnesium salts, and this fact will at once indicate its source.

Qualitative Test and Quantitative Estimation.

Special Apparatus required :

A white porcelain dish
A 100 c.c. graduated flask.
A burette graduated to $\frac{1}{10}$ths of c c.'s.
A glass stirring-rod.

Special Chemical Reagents required :

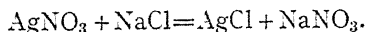
1. A cold saturated solution of the yellow chromate of potassium, which must be free from chlorine; this may be proved by acidulating a little of the solution by dilute nitric acid, and then adding a drop of nitrate of silver solution; in the absence of chlorine the solution will remain perfectly clear.

2. A standard solution of silver nitrate, made to the strength that 1 c.c. is capable of precipitating 1 milligramme of chlorine. This is made by dissolving 4·79 grammes of pure recrystallized silver nitrate in distilled water, and then making up to a litre. The solution should be kept in a brown-coloured bottle.

The reason why 4·79 grammes of silver nitrate are required to the litre of water is as follows: Cl (35·46) combines with $AgNO_3$ (169·89). Therefore 1 part of chlorine combines with $\left(\frac{169 \cdot 89}{35 \cdot 46} =\right)$ 4·79 parts of $AgNO_3$. Thus a litre of distilled water containing 4·79 grammes $AgNO_3$ will precipitate 1 gramme of chlorine, and 1 c.c. will precipitate 1 milligramme of chlorine.

QUALITATIVE TEST.

The presence of chlorine (as chlorides) may be best detected by the addition of a few drops of a solution of silver nitrate and of dilute nitric acid to the water in a test-tube, when a white haze, turbidity, or precipitate of chloride of silver will appear according to the amount of chlorine present:

$$AgNO_3 + NaCl = AgCl + NaNO_3.$$

QUANTITATIVE ESTIMATION.

1. Measure out 100 c.c. of the water in a graduated flask, and pour into a white porcelain dish.

2. Add a few drops of the solution of yellow chromate of potassium until a distinct yellow colour is furnished to the water. The object of adding this reagent is to make it serve as an "indicator," which shall denote at once the stage when all the chlorine present in the water has combined with the silver employed in the estimation.

3. The burette is charged with the standard solution of nitrate of silver, and this is added drop by drop to the water; a dull reddish precipitate of the red silver chromate forms, which, when stirred up in the water by means of a glass rod, at once disappears, owing to the chlorine in the water displacing the chromic acid and itself combining with the silver to form a white precipitate of the chloride of silver. As the addition of the standard solution is continued, the water, though it retains the yellow colour, becomes turbid, owing to the accumulation of this precipitate of silver chloride. At length a point is reached at which, there being no longer any chlorine which is not already combined with the silver, the chromic acid holds undisputed possession of this metal, and the red silver chromate remains permanently present; the first evidence of this is afforded by the yellow colour changing into a permanent orange colour ($K_2CrO_4 + 2AgNO_3 = 2KNO_3 + Ag_2CrO_4$).

Without the "indicator" there would be no means of knowing when just the amount of silver nitrate necessary to precipitate all the chlorine had been added; for it would be impossible to judge of the exact stage when the maximum amount of white precipitate of silver chloride had been created.

4. The first evidence of any red colour remaining permanent is the clue for withholding any further addition of the silver nitrate; or the amount of chlorine, estimated as it is from the amount of the solution of the silver salt used, will be over-estimated.

Example.—Five c.c. of the standard solution of silver nitrate were required to combine with all the chlorine in 100 c.c. of water, and to furnish a reddish tint to the water.

But 1 c.c. of the solution=1 milligramme of chlorine.

∴ 5 c.c. of the solution=5 milligrammes of chlorine.

∴ there are 5 milligrammes of chlorine in 100 c.c. of water.

But 100 c.c. of water=100 grammes=100,000 milligrammes.

∴ there are 5 milligrammes of chlorine in 100,000 milligrammes of water; or 5 parts per 100,000.

Conclusions to be Drawn from the Amount Estimated.—It must always be borne in mind that chlorine can only be attributed to animal organic pollution when other figures of the analysis point to the probability of such an origin. Some pure chalk and red sandstone waters furnish chlorine up to 5 parts per 100,000. In the coal-measures and certain chalk and sandstone formations, low-lying and near the coast, the water may contain up to 50 parts of chlorine per 100,000; or, on the other hand, very little indeed. Pure deep-well waters from greensand deposits may yield as much as 15 parts per 100,000, or more. Upland surface-waters free from animal pollution rarely furnish more than 1 part per 100,000.

Thresh is of opinion that as much as 100 parts per 100,000 of salt should condemn the water for drinking purposes. This amount imparts a distinct saline taste.

Notes.—Some workers deduct 0·1 c.c. for the excess of silver solution required to indicate the change of colour.

It is highly important that neither the water nor the standard solution of silver nitrate should be acid, or the results will be incorrect, since red silver chromate is soluble in an acid medium. In these cases the smallest quantity of precipitated calcium carbonate that will suffice should be added to effect neutrality.

In estimating *very* small quantities of chlorine it is advisable to first concentrate the water before titrating, as the results are otherwise very slightly in excess.

Those who have not a keen appreciation of colour change may prepare a second 100 c.c. of water, to which a precisely similar amount of the chromate has been added; if this is placed alongside the water under examination, it serves as a comparison whereby to judge the commencement of the colour change.

Coloured waters may be bleached in acid solution by means of potassium permanganate; the water is then neutralized and filtered before the chlorine is estimated.

The chlorine is sometimes expressed in terms of the common salt it is equivalent to. This is readily calculated by a comparison between the atomic weight of chlorine and the molecular weight of NaCl (common salt).

The atomic weight of chlorine is 35·46, and that of sodium is 23·00; ∴ the molecular weight of $NaCl = (35{\cdot}46 + 23{\cdot}00) = 58{\cdot}46$.

$$\therefore Cl = \frac{35{\cdot}46}{58{\cdot}46} \text{ of NaCl.}$$

Now, the chlorine in the example taken amounted to 5 parts per 100,000; then $5 = \frac{35{\cdot}46}{58{\cdot}46} NaCl$,

$$\text{or } NaCl \frac{58{\cdot}46 \times 5}{35{\cdot}46} = 8{\cdot}24.$$

∴ there would be present 8·24 parts of NaCl per 100,000 if all the chlorine present were furnished by sodium chloride. Thus the weight of chlorine $\times \left(\frac{58{\cdot}46}{35{\cdot}46} =\right) 1{\cdot}65 =$ the weight of sodium chloride.

In the State of Massachusetts it is found that the chlorine in the surface-waters and streams decreases in amount with the distance from the sea-board. The normal chlorine in waters free from all risks of pollution has been ascertained for each district, and these amounts are entered on a map of the State. Lines have been drawn connecting the districts the waters of which contain similar quantities of chlorine, and these are termed "isochlors." If the chlorine in any water is found to exceed the normal of the district from which it has been obtained, the presumption is that the water is polluted with sewage. But a chlorine test is by no means delicate enough to indicate the lesser degrees of dangerous animal contamination.

The percentage admixture of sea-water with fresh-water may be calculated from the formula $x = \frac{A-C}{C-B}$, where

x=the number of volumes of fresh-water to 1 volume of sea-water;

A=the chlorine in sea-water (which may be taken as 1,850 parts per 100,000);

B=the chlorine in the local fresh-water;

C=the chlorine in the mixture of fresh-water and sea-water.

According to Winkler, the presence of active chlorine in drinking-water which has been treated with bleaching-powder for the destruction of bacteria, is best tested for as follows: To 250 c.c. of the water are added 1 to 2 drops of a very dilute methyl-orange solution (1 : 5,000) and 2 to 3 c.c. of 10 per cent. hydrochloric acid. If hypochlorites are present, the methyl-orange is immediately, or almost immediately, decolorized, whereas a blank test on pure water remains coloured. Nitrites do not affect the test, unless present in abnormally large quantity, and even then the decolorization takes upwards of an hour.

CHAPTER IV

HARDNESS

THE " hardness " of water is of economic rather than of hygienic importance, and the main object of the estimation is to decide whether the amount of hardness is such as to render the water unsuitable for washing, cooking, and trade purposes. A hard water entails in its use a great waste of soap, for considerable difficulty is experienced in procuring a lather (1 grain of calcium carbonate will use up 8 grains of soap before a lather forms); it does not extract the same amount of strength from coffee, tea-leaves, and substances used for making soups, stews, and gravies, as softer water; and meat and vegetables boiled in it lose much of their flavour and colour, become slightly hardened and less digestible. On the other hand, moderately hard waters are always more palatable than very soft ones.

It must not be thought, however, that " hardness " in a water is a factor which can be altogether disregarded from a health standpoint, for gastro-intestinal derangement, of a degree varying with the constitution of the salts which form the " hardness," may arise among those who are constitutionally susceptible, and unaccustomed to a very hard water. The " permanent hardness " is generally mainly due to sulphates of the alkaline earths, and these have a marked aperient action when they exist in large amounts. Such waters are obtainable at Epsom, Leamington, Scarborough, and Cheltenham.

Finally, hard waters form a deposit on boilers and in pipes; and this is sometimes the cause of explosions and demands occasional removal. It is calculated that ¼ inch of the incrustation—which is a bad conductor of heat—requires the use of 45 per cent. of extra coal.

For trade purposes generally—apart from the waste of fuel, damage to boilers, and danger occasioned by the " crust " from

hard waters—it is of great importance to the process itself that the water should be moderately soft.

The factors which commonly cause the total "hardness" in water are the following:

Calcium and magnesium salts; iron, silica, and alumina; free carbonic acid, or free mineral or vegetable acids.

The "total hardness" in most of the drinking-waters of this country is largely furnished by calcium and magnesium salts, and free carbonic acid; and more especially by calcium salts.

Of the calcium and magnesium salts, the carbonates very greatly predominate as the cause of "hardness." These carbonates of calcium and magnesium, almost insoluble in pure water, are held in solution by carbonic acid, in the form of bicarbonates.

If the water be well boiled, some of the salts forming the total hardness usually become precipitated, and being no longer in solution, they cease to add to the "total hardness"; the amount of hardness thus removed is termed "temporary," and that remaining "permanent." By boiling, the carbonic acid which held the carbonates of calcium and magnesium in solution is driven off, so that these salts precipitate. Any other constituent which may have been held in solution by the carbonic acid present, such as iron, would also be precipitated. Phosphate of lime, silica, and the sulphate of lime (if present in large quantity) may also *in part* be precipitated.

The "permanent hardness" results from what still remains in solution—*i.e.*, calcium and magnesium sulphates, phosphates, chlorides, and nitrates, any iron which was not held in solution by CO_2, silica, alumina, etc. A little of the magnesium carbonate thrown down by the boiling, moreover, becomes redissolved by the time the water cools, and thus may add to the "permanent" hardness.

Although the amount of mineral solids which the water contains generally forms an index to the extent of "hardness," yet this is by no means always the case; and some saline waters yielding considerable quantities of mineral matter are "soft," a large quantity of sodium salts determining the softness.

The salts causing temporary hardness tend to furnish a loose deposit in boilers; those causing permanent hardness, a hard deposit.

QUANTITATIVE ESTIMATION.

Special Apparatus required:

A small glass-stoppered bottle of about 150 c c. capacity.
A burette with c.c.'s graduated to $\frac{1}{4}$ c.c.
A glass beaker.
Filtering apparatus.
Iron tripod, wire gauze, and triangle lined with pipeclay.

Special Chemical Reagents:

A standard solution of potassic soap or of good undried Castile soap, made to such a strength, that 1 c.c. will exactly precipitate either 1 milligramme of calcium carbonate or those other soap-destroying agents in the water to an extent which is equivalent to 1 milligramme of calcium carbonate.

Fourteen grammes of Castile soap are dissolved in a litre of a mixture of equal volumes of methylated rectified spirit and warm distilled water; it is then filtered and standardized (and, being unstable, should be restandardized every few days) by means of a standard solution of calcium chloride.

The calcium chloride solution is made by dissolving 0·2 gramme of pure crystallized calcite ($CaCO_3$) in dilute hydrochloric acid. When this is completely dissolved, evaporate to dryness on a water-bath; then add a little distilled water and again evaporate to dryness, and repeat this treatment several times to insure that all the acid has been driven off. The calcium chloride is then dissolved in a litre of distilled water. Such a solution will then contain the equivalent to 0·2 milligramme of calcium carbonate in every cubic centimetre, or 20 milligrammes per 100 c.c.

The soap solution must then either be fortified by adding a little strong solution of soap, or weakened by a mixture of water and rectified spirit (in the proportion of 3 volumes of water to 5 of spirit), until the soap solution registers hardness equivalent to 20 milligrammes of $CaCO_3$ in 100 c.c. of the calcium chloride solution.

Supposing the soap solution registers 19 milligrammes, then it is too strong, and must be weakened so as to register 20 parts—*i.e.*, if the total filtered soap solution is 990 c.c., it must be made up to $\frac{20}{19}$ of 990 c.c. = 1,042 c.c. with extra water and rectified spirit.

The *rationale* of the process is as follows: The soap employed is a combination of an alkali with a fatty acid. When it is added to water which contains calcium and magnesium salts in solution, then the fatty acids (oleic mainly in this case) will combine with the lime and magnesia to form insoluble calcic and magnesic oleate; and when the soap is added until there is no longer any lime and magnesia left to combine with, the fatty acids remaining in solution form a lather on shaking. Hence the more calcium and magnesium salts present, the larger the amount of soap solution required, and, in consequence, the longer is the production of a lather delayed.

1. One hundred c.c. of the water are placed within the small glass-stoppered bottle.

2. A graduated burette is then filled up to the 10 c.c. mark with the soap solution, of which 2 c.c. are run into the bottle, when a cloudy precipitate of insoluble calcic and magnesic oleate, etc., is formed. The bottle is then briskly shaken to see if its contents will produce a lather.

3. The solution is afterwards added in cubic centimetres, and the bottle well shaken up after each fresh addition, until eventually a certain definite amount of lather forms. The air should be sucked from the bottle (with a glass tube) from time to time, so as to remove any carbonic acid which has been liberated. Sufficient soap solution has been added when, with the bottle placed on its side, the lather presents a *thin*, unbroken surface after the lapse of five minutes. It is helpful to know that when the requisite quantity of soap solution has been added, the contents of the bottle on being shaken give only a faint, dull, soft sound; and, after shaking, small particles of the lather cling to and slowly descend the sides of the bottle.

4. From the number of cubic centimetres of soap solution required, the amount of calcium carbonate, or its equivalent (in soap-destroying power), in the 100 c.c. of water, is ascertained. But a deduction of 1 c.c. from the amount of soap solution used must be made in every case, since this amount is required to create a similar lather in the same bulk of *distilled* water—which is free from any of the ingredients which are considered as furnishing "hardness."

Example.—One hundred c.c. of water required 15 c.c. of the soap solution to furnish the characteristic lather.

Deduct the 1 c.c. which would be required for 100 c.c. of distilled water, and 14 c.c. of soap solution indicate the total hardness.

But 1 c.c. of the soap solution=1 milligramme of calcium carbonate, or its equivalent.

Therefore 14 c.c.=14 milligrammes of calcium carbonate, or its equivalent.

Therefore the "total hardness" in 100 c.c. of the water is equivalent to 14 milligrammes of calcium carbonate; and 14 milligrammes in 100 c.c. (or 100,000 milligrammes of water)=14 parts per 100,000.

Conclusions to be Drawn from the Amount Estimated.—If the "total hardness" of a water reaches 30 parts per 100,000, it

becomes unsuitable for washing and cooking purposes; and if it reaches 40 it is practically useless in these respects. A "soft" water may contain up to 10; a "hard" water from 15 to 25; a "very hard" water from 30 and upwards.

Notes.—Where the hardness exceeds 25 parts per 100,000, so much precipitate of calcic and magnesic oleate, etc., is created that it interferes with the formation of a characteristic lather, and leads to an error of over-estimation of the "hardness." In these cases it is necessary to dilute the water with an equal amount of distilled water—*i.e.*, 50 c.c. of distilled water are added to 50 c.c. of the sample, and in the estimation of the hardness 1 c.c. is still deducted from the soap solution used. The result must, of course, be multiplied by two to represent parts per 100,000.

When the results are expressed in "degrees" upon Clark's scale, 1° (Clark) is equivalent in this country to 1 grain of calcium carbonate per gallon—*i.e.*, to 1 part per 70,000. In France, however, a degree signifies 1 part of calcium carbonate in 100,000, and in Germany 1 part of lime in 100,000.

The "total hardness" having been found, the next step is to ascertain the "temporary" and the "permanent" hardness.

1. One hundred c.c. of the water are measured out and poured into a glass beaker, which is placed on an iron tripod. To protect the glass against direct contact with the flame, the beaker is placed upon a triangle lined with pipeclay, which itself rests upon a piece of iron gauze. The water is boiled until only about two-thirds of its original volume remain.

2. The mouth of the flask is covered, and its contents allowed to cool, when all the calcium and magnesium carbonate, and often the bulk of any iron present, will be contained in the precipitate noticeable at the bottom of the beaker. It is the supernatant fluid which contains the "permanent hardness," the "temporary hardness" which has been separated being represented by the deposit.

3. From the beaker the cooled water is decanted into the measuring flask, care being taken to disturb the precipitate (which is left behind) as little as possible. The water is then made up to its original bulk by filling up to the 100 c.c. mark with recently boiled distilled water; or a reflux condenser may be used while the water is boiling.

4. The 100 c.c. of water is then filtered through a fine filter-

paper and its "hardness" is estimated as previously described, and the result furnishes the "permanent hardness."

5. If the "permanent hardness" be subtracted from the "total," the difference will represent the hardness separated by the boiling—*i.e.*, the "temporary hardness."

Assuming that the permanent hardness is represented by (7—1) 6 c.c. of the soap solution, it is thus equivalent to 6 parts per 100,000 $CaCO_3$.

The total hardness was 14 parts per 100,000; ∴ the "*temporary hardness*"=14—6 or 8 *parts per* 100,000 of $CaCO_3$, or its equivalent.

Notes.—If it is desired to know the proportion of *hardness due to magnesium salts* in a water, where the "total hardness" is known to be due entirely to calcium and magnesium salts, it is necessary to first precipitate and remove all the calcium salts in the manner described in Chapter VI., when the hardness remaining will be due to magnesium salts.

Wanklyn has pointed out that whereas lime reacts immediately upon the solution of soap, magnesia requires the lapse of time; and that one equivalent of magnesia consumes as much soap solution as one and a half of lime.

If magnesium salts contribute materially to the hardness, a thin, fine, dirty scum, somewhat similar to a lather, forms upon the surface of the water as the soap solution is added. This scum finally breaks up, and is replaced by the genuine pure white lather. In such cases the water must be diluted considerably with distilled water. Bearing in mind the longer time taken for magnesia to react, the presence of this film or scum will warn the operator that as he adds the soap solution he must proceed slowly and shake thoroughly.

In Clark's softening process lime is added, in quantity depending upon the amount of carbonic acid in the water, in order that it may combine with this acid which holds the calcium and magnesium carbonates in solution ($CaCO_3,CO_2+CaH_2O_2=2CaCO_3+H_2O$). When the lime is added in excess, some of it remains in solution in the water in an uncombined state, and since this is undesirable in drinking-water, a water treated by Clark's process should be frequently tested for uncombined lime. A ready and simple method of detection is by adding a few drops of a solution of silver nitrate to some of the water, when, if free lime be present, the cloudiness created, instead of being white and clean

(silver chloride), becomes dirty and brown (an oxide of silver being formed).

The Rivers Pollution Commissioners in their Sixth Report give the following classification of waters as to their softness: (1) Rain-water; (2) upland surface-water; (3) surface-water from cultivated land; (4) river-water; (5) spring-water; (6) deep-well water; (7) shallow-well water; and they found that the following formations almost invariably furnish hard waters: (1) Calcareous silurian; (2) calcareous Devonian; (3) mountain limestone; (4) calcareous rocks of the coal-measures; (5) new red sandstone; (6) conglomerate sandstone; (7) lias; (8) oolite; (9) upper greensand; (10) chalk.

CHAPTER V

THE POISONOUS METALS

THOSE poisonous metals for which it is commonly necessary to test a water are lead, iron, and zinc.

Water most generally takes up these metals either from pipes through which it has been made to flow, from receptacles in which it has been stored, or from materials used in making or repairing the joints of pipes or cisterns; but, in addition, such metals may gain access from trade processes carried on by river-sides, or from metalliferous mines within the district, or, in the case of iron, from ferruginous soil or strata.

Lead may be taken up from the pipes and cisterns made of this material. The action of water upon this metal is primarily an oxidizing one, and in the presence of dissolved oxygen a loose coating of oxyhydrate of lead may form. The lead oxide is practically insoluble in those waters which do not contain some free acid, but when this is the case (as notably in peaty waters and those containing much free CO_2) the lead salt is carried away in solution; in other cases a relatively small quantity is removed in suspension. Ackroyd finds that plumbism due to the solvent action of peaty waters does not occur when the acidity of the water is equivalent to less than 0·5 part of sulphuric acid in 100,000 parts of water (phenolphthalein being used as indicator).

Plumbo-solvency is diminished by the presence of carbonates, sulphates, and chlorides in water which is not acid, but nitrates favour the oxidation of the metal to oxyhydrate. The action of the above salts is ascribed to the varying solubility of the lead salts of the corresponding acids, the nitrate being the most soluble, and the sulphate and carbonate the least so. Since non-acid waters containing carbonate of calcium provide a coating of carbonate of lead to the surface of the metal, and this coating is insoluble in such waters, unless there is free CO_2 over and above

the amount necessary to form bicarbonate, it follows that soft waters are the great lead-carriers. Soluble phosphates in the water will also protect the metal to a marked degree. As a general rule, then, soft waters attack and hard waters protect lead; but in certain districts hard waters containing free CO_2, but a small amount of carbonate, are capable of dissolving appreciable amounts of lead.

"Soda-water" is particularly liable to take up large quantities of lead if it is allowed to come into contact with that metal.

Waters containing a mere trace of lead often present a faint haziness. This disappears on adding nitric acid.

Iron.—A chalybeate water generally contains its iron in the form of ferrous carbonate held in solution by an excess of carbonic acid; on prolonged exposure to air, or by applying heat, hydrated ferric oxide, or "rust," is thrown down ($4FeCO_3 + O_2 + 2H_2O = 2Fe_2O_3H_2O + 4CO_2$), since it is insoluble in water containing no free acid. Upland, moorland, and some other waters (as those from the greensand and new red sandstone) generally contain traces of iron, which are taken up from the soil or strata permeated.

The solution of iron from soils is generally due to organic matter removing oxygen, and thus converting the iron to the ferrous condition, in which form it is soluble in water containing carbonic acid.

Copper is rarely found in drinking-water; but it is sometimes given to water by culinary utensils made of this metal, for a small amount of copper is dissolved when water which contains common salt, acid (vinegar, etc.), fatty or oily material, is boiled in contact with it. The writer has recorded an instance where the practice of placing a penny into the saucepan in which vegetables are boiled, in order to improve their colour, gave rise to symptoms of copper-poisoning in two children of a household.

Zinc is most generally taken up from galvanized iron cisterns and pipes or zinc surfaces. All kinds of water attack zinc in the presence of air, even hard waters with an alkaline reaction. But it generally exists in water in the form of carbonate held in solution by CO_2, and, being held in solution as a bicarbonate, zinc is completely thrown down by continued boiling. As with water containing copper, the bacterial count is low after twenty-four hours' standing. Galvanized iron must not be held to entail danger in its use, unless the water contains much free carbonic acid,

since under common conditions the zinc oxide or basic carbonate form and protect the metal from further action. Zinc, as sulphate, has been observed in considerable quantity in certain springs in the South of France, New Zealand, and America.

Chromium.—Chromium may possibly get into water from colour and dye works, etc., but it is extremely rare that this very poisonous metal ever gains access to drinking-water.

Tin, arsenic, and **barium** are rarely found in water; but **manganese** is not uncommonly found in some parts of the Continent.

QUALITATIVE TESTS AND QUANTITATIVE ESTIMATION.

Special Apparatus required:

White porcelain basins.
Boiling flask.
Burette with c.c.'s graduated to tenths of c.c.
Nessler glasses.
Filtering apparatus.
Ignition crucible and crucible tongs.
Desiccator.
Drying oven.
Chemical balances.
Wash-bottle.
Marsh's apparatus.

Special Reagents required:

1. A standard solution of lead acetate—1 c.c. = 1 milligramme of lead—made by dissolving 1·83 grammes of crystallized acetate of lead in a litre of distilled water.

2. A standard solution of copper sulphate—1 c.c. = 1 milligramme of copper—made by dissolving 3·927 grammes of sulphate of copper in a litre of distilled water.

3. A standard solution of ferric chloride—1 c.c. = 1 milligramme of iron—made by dissolving 1·004 grammes of iron wire in nitro-hydrochloric acid, precipitating with ammonia solution, washing and redissolving the ferric oxide in a little hydrochloric acid, and then diluting to a litre.

Each of these standard solutions may be diluted, in some cases with advantage, so that each c.c. contains 0·1 milligramme of the metal.

Solutions of—
Sulphuretted hydrogen.
Cyanide of potassium.
Ferrocyanide of potassium.
Yellow chromate of potassium.
Ammonia.
Ammonium chloride.
Mercuric chloride.
Peroxide of hydrogen.
Dilute hydrochloric acid.
Solid potassium nitrate and sodium carbonate.
Granulated zinc.
Metallic copper.

Qualitative Tests.

Though the metals lead, copper, and iron, even when existing in faint traces, may generally be detected by testing the original water, it is sometimes desirable to reduce the bulk of the water—and thus concentrate their solutions, as it were—by evaporation before testing. In the case of zinc, it will *always* be necessary to thus considerably reduce the original bulk of the water. A litre of water may be evaporated to 200 c.c. by previously marking a narrow beaker at the precise level to which 200 c.c. of water reaches, and, after acidulating with a drop or two of hydrochloric acid, in order to keep the metals in solution,* boiling the litre of water until it is reduced to this level.

Lead, Copper, and Iron.—1. To about 100 c.c. of the sample of water, placed in a white porcelain dish, apply a little of the sulphuretted hydrogen solution by means of a glass rod which has been dipped in the solution. By drawing the rod gently through the water, and noticing any discoloured streak immediately adjacent to the track of the rod, faint quantities of poisonous metals will be more readily detected than by allowing a drop of the reagent to fall into the water and then stirring. The reason for this is that the reagent itself may impart a faint colour, and it is therefore advisable to add as little of it as possible to commence with, otherwise a faint discoloration caused by a metal may be lost in that created by the reagent.

Any evidence of a dark colour appearing in the water denotes the formation of the sulphides of either lead, iron, or copper, and the sulphuretted hydrogen should then be further added until the *maximum* amount of darkening has been produced. It must be borne in mind that iron when in faint traces may only impart a slight dirty green colour to sulphuretted hydrogen at first, but after a while the black colour of the sulphide forms.

If there is any colour present in the *original* water, a comparison must be made with a similar quantity of the water placed in another porcelain dish before it is decided whether any additional colour has been furnished by the sulphuretted hydrogen. Where, however, the colour originally present is marked (as by peat, etc.), it may well obscure, even with these precautions, a trace of lead, iron, or copper. It must then be decolorized as follows: 100 c.c. are acidified with hydrochloric acid and heated to

* If the presence of lead is suspected, the water should not be acidulated.

boiling; a crystal of sodium chlorate is added to the liquid, which is next boiled until the excess of chlorine is expelled. Ammonia is then added to the cold solution until it is just alkaline, and the whole diluted to its original volume with distilled water.

2. If the water darkens, pour half of it into a second porcelain dish. To one part add a drop or two of dilute hydrochloric acid, when if the colour disappears it is due to *iron*; or if it diminishes perceptibly iron is present.

3. A confirmatory test should then be applied to some of the water in a test-tube—*i.e.*, a drop or two of HCl is followed by a few drops of a solution of the ferrocyanide of potassium, when the colour of Prussian blue (ferrocyanide of iron) is produced. Another very delicate test is to boil the water with a few drops of nitric acid, cool, and add a little potassium sulphocyanide, when a blood-red or sherry colour results, due to ferric sulphocyanide.

4. If, after adding the hydrochloric acid, the colour does *not* disappear, the metal is either *lead or copper*. To the other half of the darkened water add a few drops of a solution of potassium cyanide; the PbS will be unaffected, but CuS will be completely dissolved.

An excellent confirmatory test for lead is to add to some of the water in a test-tube a few drops of a solution of the yellow chromate of potassium, when if lead is present an opacity appears in the water (due to the formation of lead chromate). The reaction, is, however, difficult of appreciation with faint traces of lead, which will often be missed by this test unless a careful comparison is instituted with another test-tube containing a similar amount of lead-free water and reagent.

Iron may possibly be present along with lead, and may contribute to the darkening created by the sulphuretted hydrogen. If so, this may be detected by adding a drop of dilute hydrochloric acid to the water, for this has been seen to remove any darkening furnished by an iron salt. Or the iron may be separated by adding nitric acid, evaporating to a small bulk, and precipitating the iron with excess of ammonia and warming; the precipitate of ferric oxide may be separated on a filter-paper, washed, dissolved in nitric acid, then reprecipitated with ammonia, and again filtered and washed; the filtrate should be boiled until the ammonia is driven off, and then tested for lead.

5. As a confirmatory test for copper, a drop or two of a solution of the ferrocyanide of potassium should be added to some

of the water after it has been acidulated with a drop of dilute hydrochloric acid. If copper is present, a bronze coloration and precipitate of cupric ferrocyanide appears.

A faint colour will often be missed, unless it be looked for through the depth of the water on to a white background.

6. When no darkness is created and it is judged desirable to test for **zinc,** concentrate the water; render slightly alkaline with ammonia; add a few drops of ammonium chloride solution; then boil; add to some of the further concentrated water, after filtration, a few drops of ammonium sulphide. The white precipitate (of hydrated sulphide) formed in the presence of zinc, is very characteristic, since it is of a flocculent, curdled, or gelatinous nature.

As a confirmatory test, render the water slightly alkaline with ammonia; further concentrate by boiling; filter; add a few drops of the ferrocyanide of potassium with excess of dilute hydrochloric acid, and note a white gelatinous precipitate of zinc ferrocyanide, insoluble in dilute acids. Potassium ferricyanide furnishes a rusty yellow precipitate of zinc ferricyanide, soluble in hydrochloric acid and ammonia.

7. The presence of **arsenic** has extremely rarely to be tested for, but when it is desirable to do so Marsh's test is the most delicate.

A litre of water is rendered alkaline by solid sodium carbonate (free from arsenic); evaporated nearly to dryness; and the residue introduced into Marsh's apparatus. For a full description of the application of Marsh's test, see "Arsenic in Food."

8. **Tin.**—A litre of water should be evaporated to a solid residue, and the tin dissolved out from the ash by warming with some strong hydrochloric acid; then dilute a little and boil for a *long* time with metallic copper to make certain that the tin exists in a stannous condition; decant and add excess of a solution of mercuric chloride, when a silky-looking cloud of mercurous chloride appears ($2HgCl_2 + SnCl_2 = SnCl_4 + 2HgCl$). If a mixture of ferricyanide of potassium and ferric chloride be added to a solution containing stannous oxide or chloride and hydrochloric acid, a precipitate of Prussian blue results from the reduction of the ferri- to the ferro-cyanide. If no other reducing agents are present, this is very delicate. Sulphuretted hydrogen yields a dark brown precipitate with stannous salts, soluble in potassic hydrate.

9. **Chromium.**—A good test is to collect the residue from a litre of water; fuse the ash with solid potassium nitrate and sodium carbonate, so as to produce the yellow chromate of potassium; this in a neutral solution yields a purple precipitate with excess of silver nitrate. Slight traces may be detected by concentrating the water to a very small bulk, and then letting it drop upon a thin layer of ether floated on a dilute solution of peroxide of hydrogen acidified with sulphuric acid; the blue colour that forms in the lower solution passes over to the ether upon slight agitation.

10. **Manganese.**—Occasionally this metal is found in water, and its presence has been noted more particularly in America and Germany. A delicate test (Wanklyn) is to evaporate a litre of water to a small bulk; nearly neutralize with hydrochloric acid; and treat with a few drops of peroxide of hydrogen solution, when a brown precipitate forms in the presence of manganese. The oxidation of manganese to permanganic acid in the presence of sulphuric acid and a drop of nitrate of silver solution as the oxidizing agent, is equally sensitive.

Having thus detected the presence of a poisonous metal, it becomes necessary to estimate its amount.

Quantitative Estimation.

The estimation of lead, copper, and iron may be performed by a colorimetric or colour-matching process.

1. Measure out 100 c.c. of the concentrated water which has been found to contain **lead,** and pour into a Nessler glass (a glass cylinder graduated to 50 c.c.).

2. Place a similar amount of lead-free water into three other Nessler glasses, to which different amounts (from 0·1 to 1·0 c.c.) of a standard solution of the metal have been carefully added.

3. To each glass add one drop of the sulphuretted hydrogen solution, and well stir with a glass rod reserved for each basin.

4. Note which of the standard waters forms a match with the water under examination, and therefore contains the same amount of Pb.

Example.—The amount of standard solution added to the particular 100 c.c. of distilled water which matched the brown coloration in the sample of lead-polluted water was 0·4 c.c.

But 1 c.c.=1 milligramme of lead.

∴ 0·4 c.c.=0·4 milligramme of lead.

∴ there is 0·4 milligramme of lead in 100 c.c. of the concentrated water.

But the water was concentrated to one-fifth of its original bulk; ∴ the 0·4 milligramme of lead represents 0·08 milligramme in 100 c.c. of the original water, or 0.08 part per 100,000, or 0·056 grain per gallon (about $\frac{1}{18}$ grain). It is often better to work with a weaker standard solution made up to one-tenth of the strength of the solution here referred to; and as it is only very light shades of brown which can be matched with great precision, if there is much metal present it may be necessary to lessen the degree of concentration, or even to deal with the original water.

It may be noted that if lead furnishes any evidence of its presence by tests applied to the original water, the metal is present in harmful amount.

Similarly, a quantitative estimation of **copper** and **iron** may be made by employing standard solutions of these metals.

Iron may be estimated gravimetrically, if in appreciable amount, in the following manner, and the colorimetric estimation thereby checked:

The ash from the residue of 500 c.c. of the water is digested in strong hydrochloric acid; after filtration, the filter-paper and its contents are well washed with boiling distilled water; then add two drops of nitric acid to the filtrate, boil, and add ammonium chloride solution and a slight excess of ammonia. Collect the precipitate on a Swedish filter-paper; wash with boiling water; dry in hot-air oven at 105° C. The filter-paper should next be folded up, placed in a small porcelain crucible (previously weighed) and covered by a lid; then ignite to dull redness, at first gently so as to obviate spurting and loss, and the lid should be removed after a while so as to permit free access of air. When the filter-paper has been entirely destroyed, let the capsule and its contents cool under a desiccator, and weigh. The weight found, minus that of the crucible and the ash of the filter-paper, represents the weight of Fe_2O_3, and this $\times$ 0·7=Fe.

The quantitative estimation of **zinc** can be conveniently made, gravimetrically, by taking a measured quantity of the concentrated water (which is found to be free from other poisonous metals) and collecting the precipitate of zinc sulphide (obtained

as described above) on a filter; this is then well washed with dilute ammonium sulphide, dried, ignited in a weighed capsule at a bright red heat, allowed to cool, and finally weighed as oxide (ZnO). The weight obtained $\times$ 0·8 = Zn. Or an approximate estimation may be made by preparing a standard solution of zinc (4·4 grammes of the sulphate to 1 litre of water; 1 c.c. = 1 milligramme Zn), and matching, on the lines indicated above, the turbidity produced in 100 c.c. of the concentrated water after a small measured quantity of potassium ferrocyanide has been added.

For the determination of small quantities of **manganese** in drinking-water, the colorimetric method of Volhard and Treadwell, modified for drinking-water, is recommended—that is, oxidation of manganese to permanganic acid with nitric acid and lead peroxide, and comparison of the coloured liquid with nitric acid solutions containing known amounts of permanganate.

A very approximate quantitative estimation may be made, colorimetrically, as follows (Haas):

One hundred c.c. of the water under examination are acidified with 5 c.c. of 20 per cent. sulphuric acid, 1 gramme of potassium persulphate is added, and the solution is heated until a reddish-violet coloration is obtained, or until a brown colour, due to manganese dioxide, develops. The solution is now cooled, a trace of sodium hydrogen sulphite is added, and the oxidation with persulphate is repeated. The coloration obtained is then compared with that exhibited by $\frac{N}{100}$ potassium permanganate solution.

Conclusions to be Drawn from the Amount Estimated.—Opinion is somewhat divided as to the amounts of the poisonous metals which may be considered dangerous in drinking-water. In the case of **lead,** $\frac{1}{20}$ of a grain to the gallon is accepted as the limit by many authorities; for in the case of a poisonous metal which is cumulative in its action, a very small trace should be considered sufficient to render condemnation of the water justifiable.

There is evidence that the system becomes habituated to **copper** salts, but this metal, if allowed at all, certainly never ought to exceed $\frac{1}{15}$ grain to the gallon in drinking-water, for it is cumulative—though to a less degree than lead.

Zinc is not harmful to the same extent. The writer has found up to 1 grain per gallon of the metal (as carbonate) in many waters which have been in contact with galvanized iron,

and used for many years in public institutions without any evidence of harm. Others have found even more in water which has been drunk without harmful consequences. Scott and Jameson record (*Lancet,* June 30, 1917) that for two years 200 men drank water containing from 1·7 to 3·3 grains per gallon, without any disturbance of health. But individual toleration varies, and the amount found is probably increased at some other time or season, and so $\frac{1}{3}$ grain per gallon should not be exceeded.

The faintest trace of **arsenic**—an exceptionally poisonous and somewhat cumulative metal—would suffice to condemn the water.

Iron, since it gives indication of its presence when in such amounts as would make its ingestion undesirable, by imparting a distinct taste to the water, has rarely led to harmful results; for people will not, as a rule, adopt a water-supply that is unpleasant to the palate. A quarter of a grain per gallon is an amount which is just appreciable by taste. But iron in a general water-supply should not exceed $\frac{1}{4}$ to $\frac{1}{2}$ grain per gallon, as excess of this quantity may after a time provoke dyspepsia, headache, etc., in some people.

To determine the solvent action of a particular water on lead or zinc surfaces, a piece of the clean metal may be submerged for twenty-four hours in a known quantity of the water in question.

CHAPTER VI

CALCIUM AND MAGNESIUM SALTS—SILICA—SULPHATES—PHOSPHATES

Special Reagents required:

Solutions of—
- Ammonium chloride.
- Ammonia.
- Ammonium oxalate.
- Sodium phosphate (saturated).
- Barium chloride.
- Hydrochloric acid.
- Nitric acid.

Molybdic Solution.—Dissolve 1 part of pure molybdic acid in 4 parts of NH_3 (S.G. 0·960); filter, and pour with constant stirring into 15 parts of nitric acid (S.G. 1·2); let stand in the dark for a few days; carefully decant and keep in the dark.

Magnesium Mixture.—Fifty-five grammes of crystallized magnesium chloride are added to 70 grammes of ammonium chloride, and the whole dissolved in 1 litre of 2½ per cent. ammonia. About 15 c.c. of the mixture should be used to precipitate 0·1 gramme P_2O_5.

Special Apparatus required:

- Filtering apparatus.
- Platinum dish.
- Desiccator.
- Ignition crucible and tongs.
- Glass beakers and stirring rods.
- Water-bath and drying oven.

CALCIUM SALTS.

The presence of calcium salts, which mainly exist as the bicarbonate and sulphate in water, may be indicated as follows: Add a solution of ammonium chloride and sufficient ammonia to furnish a slight ammoniacal odour. If there is any opacity or precipitate (due to ferric hydroxide, etc.), filter; then add ammonium oxalate solution to the filtrate, when a white precipitate of calcium oxalate forms. The ammonium chloride serves to

hold any magnesium oxalate in solution, as soluble ammonio-magnesium oxalate.

For a **quantitative estimation,** a measured quantity of water (say 200 c.c. concentrated from a litre of water previously acidified with a drop or two of HCl) must be thus treated and set aside in a warm place for several hours; the precipitate carefully filtered (until the filtrate is quite clear) through a Swedish filter-paper; the oxalate of calcium precipitate remaining on this filter-paper is thoroughly washed with hot distilled water, and afterwards dried in the hot-air oven at a temperature of 105° C.; it is then ignited, the capsule and its contents allowed to cool under the desiccator, and the weight taken. The weight found, minus that of the crucible and the ash of the filter-paper, represents the weight of the calcium as carbonate—to which form the oxalate has been reduced by ignition.

MAGNESIUM SALTS.

These generally exist in water in the form of the bicarbonate and sulphate, and chiefly in water collected from sandstone deposits in the neighbourhood of the coast and from the dolomite strata. If these salts exceed 10 parts per 100,000 they may cause dyspepsia and diarrhœa in those unaccustomed to the use of such waters. The presence of magnesium salts may be best ascertained by precipitating all the lime present in the water by means of a solution of ammonium oxalate, ammonium chloride, and ammonia; filtering until the filtrate is perfectly clear and free from lime, as shown by ammonium oxalate solution furnishing no opacity; the filtrate, slightly acidified with hydrochloric acid, should next be concentrated by boiling, and a few drops of a saturated solution of phosphate of sodium added, with sufficient ammonia to create strong alkalinity; the whole is well stirred up with a glass rod and then set aside for several hours, when a crystalline precipitate of a double phosphate of magnesium and ammonium (ammonium-magnesium phosphate) is formed.

In those cases where the magnesium salts are present only in minute traces no definite precipitate results, but the points where the stirring-rod has touched the glass appear as white streaks, readily soluble in hydrochloric acid.

The above precipitate of ammonium-magnesium phosphate may for the purpose of a **quantitative estimation** be collected on

a filter; washed with dilute ammonia; dried; ignited at a red heat; and weighed when cold as pyrophosphate ($Mg_2P_2O_7$), to which the red heat reduces the double salt. $Mg_2P_2O_7 \times 0{\cdot}219 = Mg$.

The amount present can also be approximately estimated from the hardness which magnesium will create when a water, previously freed from calcium salts, is tested by the soap solution. Supposing, for instance, 5 c.c. of the soap solution are required to satisfy the hardness remaining in 100 c.c. of such water; deduct 1 c.c. (the amount of solution required to produce a similar lather in an equal bulk of distilled water) = 4 c.c.

But 1 c.c. of soap solution = a milligramme of calcium carbonate.

∴ 4 c.c. = 4 milligrammes of calcium carbonate.

∴ the hardness due to magnesium salts in 100 c.c. of water is equivalent to 4 milligrammes of calcium carbonate. But 1 part of calcium carbonate is equivalent to 0·56 part of magnesium carbonate (Wanklyn), since 1 equivalent of magnesia consumes as much soap as $1\frac{1}{2}$ equivalents of lime; for $CaCO_3$ is to $MgCO_3$ not as their respective molecular weights (*i.e.*, 100 to 84), but as 150 : 84, or as 1 : 0·56.

∴ the magnesium would be equivalent to $4 \times 0{\cdot}56 = 2{\cdot}24$ *parts of magnesium carbonate in* 100,000 *of water, or* 1·56 grains per gallon.

Magnesium carbonate has been estimated as high as 9 grains per gallon by Wanklyn and Playfair, in a Sunderland water.

SILICA.

The estimation of silica may become of importance, having in view the fact that its presence diminishes the plumbo-solvent action of water.

For the **quantitative estimation** a measured quantity of water, say 500 c.c., is slightly acidulated with hydrochloric acid, and then evaporated to a solid residue; this is treated with strong hydrochloric acid, and afterwards well washed with boiling distilled water; the residue collected on a filter is dried, ignited, and again treated with the acid and washed as before; any residue ultimately left will consist of most, if not all, of the silica originally present in the water, and the white gritty powder of silica (SiO_2) may be dried, ignited, and weighed.

SULPHATES.

Sulphates exist in most waters, especially those which have been in contact with selenitic* deposits. They are either derived from the soil or strata over or through which the water has passed, or from the sulphur contained in organic pollution (urine, etc.). The rain-water collected in large towns yields small amounts originally derived from the sulphur in the coal burnt. Sulphates in water sometimes result from the oxidation of metallic sulphides (chiefly iron pyrites), which exist as such in certain deposits. They mainly exist as calcium and magnesium sulphates, and less generally as sodium sulphate; and either of these salts, if present in large amount, would tend to cause diarrhœa and dyspepsia in those unaccustomed to the use of the water.

Waters collected from the limestone and dolomite formations always contain a marked amount of sulphates. The sulphates in limestone may reach to 20 parts per 100,000, and consist mainly of calcium sulphate; while those in magnesium limestones and dolomite consist partly of magnesium sulphate. Chalk waters are relatively poor in sulphates.

The varieties of growth found capable of fixing sulphur and flourishing when sulphuretted hydrogen and sulphates are abundant in water (as in the case of gross sewage contamination and acid waste-waters gaining access) are: *Leptomitus lacteus, Sphærotilus natans, Beggiatoa alba,* and certain *zooglea* masses which may assume a branching appearance and simulate the other forms microscopically. Each form presents to the naked eye the appearance of long dirty white tufts, which are attached to stones, etc., in the bed and on the banks of streams below the level to which the water reaches.

Qualitative Test.—To some of the water previously acidified with dilute HCl and placed in a test-tube, a few drops of chloride of barium solution are added; this is then left to stand for a few minutes, when an opacity or precipitate of the sulphate of barium is created with even very small quantities of sulphates ($H_2SO_4 + BaCl_2 = BaSO_4 + 2HCl$).

For the **quantitative estimation,** 100 c.c. of water (concentrated from a litre) should be strongly acidified with hydrochloric acid, heated to boiling, and an excess of a hot 3 per cent. solution of

* Selenite is a natural foliated or crystallized sulphate of lime.

barium chloride cautiously added, with constant stirring, until the maximum turbidity is furnished; the precipitate formed is collected on a small Swedish filter-paper, washed, ignited at a moderate red heat, and weighed as barium sulphate; the washing of the precipitate is continued until the filtrate no longer gives a turbidity with silver nitrate.

If there is any doubt as to whether sufficient of the barium chloride solution has been added, let stand until the barium sulphate has settled, then add more barium chloride solution to the clear supernatant water, and note if any further turbidity occurs. If not, sufficient has been added.

To express the result in terms of sulphuric acid (SO_3) it is necessary to multiply the weight of barium sulphate by 0·343. In drinking-waters the amount of SO_3, as sulphates of the alkalies and of magnesium, should not exceed 10 parts per 100,000.

PHOSPHATES.

The phosphates found in water are commonly those of potassium, sodium, and ammonium, and their double salts. Their presence affords corroborative evidence of animal contamination (especially urine); but they may only exist in small amounts in waters dangerously polluted, for phosphoric acid is eagerly retained by the soil which the water may have subsequently come in contact with. When this point is considered in conjunction with the facts that traces of phosphates may also have their origin in strata—chiefly sandstone—permeated, and that they have also been found to be present in some marshy waters unpolluted with animal matter, it will be realized that they do not often furnish evidence of value to the analyst. But when in marked amount they may be taken as a certain sign of dangerous organic pollution. Their complete absence, on the other hand, is no guarantee of a water's freedom from such pollution.

In every case before a test is applied for phosphates the water should be reduced from a large bulk to a very small one by evaporation, and it is even preferable to dissolve the phosphates out from the ash of the water.

Qualitative Test.—Five hundred c.c. of water are acidified with a little nitric acid and evaporated to a solid residue; the residue is dried over a water-oven for two hours, to render any silica

insoluble; then dissolve in 3 c.c. of dilute nitric acid and filter; mix the filtrate with 3 c.c. of molybdic solution, gently warm, and set aside for fifteen minutes at a temperature of about 26° C. If "traces" of phosphates are present, a faint greenish-yellow turbidity will be noted; if "heavy" traces, a marked yellow precipitate falls.

The **quantitative estimation** may be made by comparing the colour results obtained with standards made by diluting varying quantities of a standard solution of sodium phosphate (1 c.c.=0·1 milligramme of P_2O_5); or if the precipitate (which consists of yellow phospho-molybdate) is appreciable, it may be collected from 500 c.c. of water, washed with distilled water, dissolved in ammonia, and precipitated with magnesium mixture. This precipitate is then collected and washed with 2½ per cent. ammonia, ignited, and weighed as $Mg_2P_2O_7$ (magnesium pyrophosphate). The $Mg_2P_2O_7 \times 0{\cdot}64 = P_2O_5$. If this is the amount in 500 c.c. of water, one-fifth of this will represent parts per 100,000.

More than 0·05 part of P_2O_5 per 100,000 should always be regarded with suspicion (Hehner).

CHAPTER VII

THE SOLID RESIDUE

Special Apparatus required :

Water-bath.
Platinum dish.
Drying oven.
Crucible tongs.
Chemical balances.

By " the solid residue " of water is generally implied the substances which are held in solution, and which, when the water is evaporated to dryness, remain behind; and such a significance must be attached to the expression " total solids " throughout Part I. of this book.

The solubility of much of the matter taken up by water may be determined by soil micro-organisms, and the amount of solid matter in water collected from a depth will depend upon the geological characters of the locality from which it has been collected.

The Process.

The suspended matters are first allowed to subside, or are separated by filtration either through a clean porcelain filter or through several large filter-papers, ribbed, and packed rather loosely into a large funnel. These filter-papers must be previously well washed with distilled water.

1. Fifty c.c. of the water are placed in a previously weighed platinum dish; this is then put upon the water-bath, and it may be protected from dust by means of a small glass cover, one side of which is raised a little by inserting a glass rod beneath it, so as to allow the condensed moisture to escape.

2. When the water is evaporated to apparent dryness, the dish is removed and placed for half an hour in the hot-air oven, in order that the "solid residue " may be finally dried at 105° C.,

the object being to remove all adventitious moisture, but not the water, which is an essential constituent of the substance as "water of crystallization."

3. The dish is removed from the oven, and then allowed to cool under a desiccator.

4. In fifteen minutes the dish and its contents are weighed, and the difference between the weight found and that of the clean and empty dish represents "the total solids" in 50 c.c. of water.

5. By means of a pair of platinum-pointed crucible-tongs the dish is next held in the flame of the Bunsen burner and *slowly* heated to dull redness, when any organic matter will give evidence of its presence by charring. If in small amount, this charring only causes an evanescent brown shade of coloration to spread over the residue; but if large quantities are present, the organic matter during incineration shows blackened specks or patches, which slowly disappear and give off dark fumes which may possess an odour of burnt hair or horn when due to nitrogenous animal matter, or of burning sugar if the material is vegetable. When a large amount of oxidized compounds of nitrogen exist, they may give rise to an evolution of red fumes of nitrogen dioxide. Marked scintillation is sometimes also perceptible—that is to say, tiny sparks are emitted. As the result of this ignition, eventually nothing remains but clear *white* or *grey* mineral ash, except where iron is markedly present and imparts a reddish tint to the ash.

6. The dish is allowed once more to cool under the desiccator and is reweighed; then the excess of weight over that of the clean and empty dish consists solely of *mineral* ash, and represents the "non-volatile solids."

7. The weight of the total solids, less the weight of the "non-volatile solids," represents the "volatile solids."

Example.—The clean platinum dish weighs 44·225 grammes.

The dish + the total solids weigh 44·245 grammes.

∴ 44·245 − 44·225 = 0·020 gramme of total solids in 50 c.c. of water, or 0·040 gramme in 100 c.c. (100 grammes).

∴ there is 0·040 gramme of total solids in 100 grammes of water.

Or 40 *parts per* 100,000 *of total solids.*

After ignition the dish + contents weigh 44·240 grammes.

∴ the "non-volatile solids" in 50 c.c. water = 44·240 − 44·225 = 0·015 gramme.

∴ there is 0·030 gramme in 100 grammes of water.

Or 30 *parts per* 100,000 *of "non-volatile solids."*

Thus the total solids amount to 40 parts and the non-volatile solids to 30, and the difference of 10 *parts per* 100,000 will represent the *volatile solids*.

Notes.—It may be pointed out that a few drops of dilute hydrochloric acid will, by creating little or much effervescence, roughly indicate the amount of carbonates present, and will generally dissolve out everything but silica and the sulphates of calcium and magnesium.

In the case of mineral medicinal waters and those used for some trade purposes, a detailed and complete analysis of the mineral ash may be required; but for public health purposes, in view of the information which is acquired in other steps of the analysis, a complete analysis of the ash is not necessary. This matter is therefore beyond the scope of this work; but it may be stated that in regard to the principles which guide chemists as to the association of the different acids and bases to form the saline matter in water, it is assumed that the combinations are governed by their respective affinities—that is to say, the strongest acid is assumed to be combined with the strongest base, due attention being also paid to the greater or less degree of solubility of the salts, since it is well known that this exercises a considerable influence on the manifestations of the force of affinity.

Thus it is assumed that the chlorine is combined with sodium, any excess being allotted to potassium, or, in the absence of potassium, to calcium. If there is excess of sodium, it and the potassium are assumed to be in combination with sulphuric acid, any excess of which is allotted to calcium and magnesium; and calcium and magnesium, if not combined with sulphuric acid, nitric acid, or chlorine, are in the form of bicarbonates.

Nitric acid is held to be combined with ammonia, and when there is excess it is allotted to either soda or lime or magnesia (as circumstances indicate) in waters which are found to be comparatively free from organic matter; otherwise it may be allotted to fixed organic bases.

The other constituents of the mineral residue, being in such small amounts, are generally not grouped as salts, the silica, iron, alumina, nitrous acid, and phosphoric acid being returned as SiO_2, Fe_2O_3, Al_2O_3, N_2O_3, and P_2O_5, respectively.

To give a simple example:

A mineral water is analyzed and found to contain:

	Parts per 100,000.
Sulphuric acid	186·07
Soda	66·72
Magnesia	52·76
Chlorine	13·40
Lime	6·68
Carbonic acid	2·12
	327·75

These constituents would be expressed in combination as follows:

Sulphate of magnesium	158·28
Sulphate of sodium	132·86
Sulphate of calcium	9·69
Chloride of sodium	22·11
Carbonate of calcium	4·81
	327·75

The ignited residue may be retained, as a routine practice, for the estimation of phosphoric acid. A loose white light residue indicates the presence of magnesium.

Most good waters furnish a solid residue, which, when ignited, shows no darkening; but the solids of potable peaty waters may show marked charring.

Surface-waters generally furnish from 5 to 20 parts of total solids, according to the nature of the surface; well-waters from 20 to 60, or over.

The total solids have been estimated even above 300 parts per 100,000 in certain deep-well waters.

A high amount of mineral solids, consisting as it so frequently does of harmless salts (such as calcium carbonate), is not necessarily injurious; but if a goodly proportion of the mineral residue is found to be contributed by sulphates, the water may be productive of digestive disturbances; and, generally speaking, the mineral matter in a domestic water-supply should not exceed 100 parts per 100,000.

CHAPTER VIII

THE EXAMINATION OF SUSPENDED AND DEPOSITED MATTER IN WATER

Chemical.—The estimation and examination of the "total solids" have only included those solids which are in solution, but some samples of water contain suspended matter and sediment.

The most simple method by which the matter which will **deposit** can be collected and estimated is the following: After well shaking, remove a litre of the sample; place it in a large conical flask; cover up and set aside for twenty-four hours; decant or siphon off as much of the supernatant water as it is safe to do without running any risk of disturbing the deposited matter; the deposit should then be shaken up with about 200 c.c. of distilled water, and after deposition that remaining behind in the flask should then be washed by distilled water into a platinum dish, dried, and weighed.

The sediment may then be carefully incinerated at as low a temperature as possible, and the volatile and non-volatile matter estimated.

FIG. 10.—WYNTER BLYTH'S TUBE FOR COLLECTING SEDIMENTS.

Wynter Blyth's tube is a convenient instrument for collecting water sediments; as seen in Fig. 10, it is similar in appearance to a large pipette capable of holding a litre of water. A small glass cell fits over the small lower extremity of the tube, and into this the deposited matter gradually collects. After the insertion of the long rod-shaped stopper, which plugs the outlet to the tube, the cell can easily be removed

without the sediment being disturbed. This may then be transferred to a platinum dish, dried, and weighed.

Recently washed and ignited fine quartz sand may be employed to filter off the material for microscopic examination; all the filtered material from a large bulk of water thus collected may be subsequently washed out by a little distilled water and examined.

Where a sediment forms from a sample of water, advantage should be taken of the valuable evidence as to its nature which

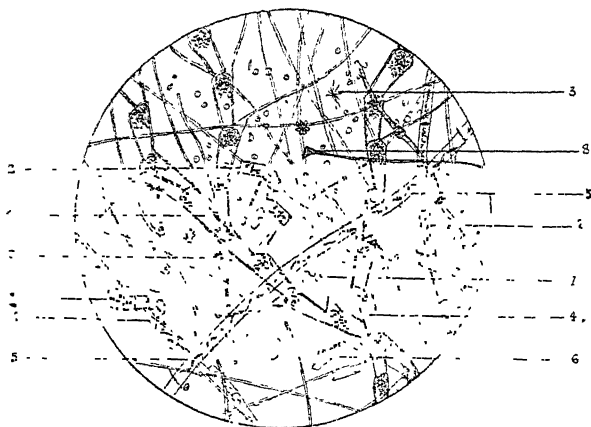



FIG. 11.—SHOWING THE SEDIMENT OF A POND-WATER, A SAMPLE OF WHICH WAS COLLECTED IN THE EARLY SPRING (× 250). DRAWN BY A. E. EVANS, M.B.

1, A desmid; 2, *Tabellaria floccosa* (Diatomaceæ); 3, *actinophrys*; 4, a confervoid growth; 5, a vegetable spiral vessel; 6, silicious particles; 7, conferva; 8, *gomphonema*. Various forms of minute unicellular plants are seen scattered about the "field."

a **microscopic examination** affords. Some of the sediment may be taken up by means of a small pipette, and transferred to several glass slides and cover-glasses applied, any excess of water upon the slide being removed by clean blotting-paper; or, better still, the sediment may be collected by means of centrifugalization, and the deposit from the centrifuge may be mounted and examined. When **suspended** matter is so light that it will not settle, drops of the water must be examined under the microscope.

If a quantitative estimation is desired, the water should be

allowed to rest for twenty-four hours, so as to exclude matter which will deposit, and the total solids in the supernatant water are then estimated; the solids remaining should be ascertained after the same water has been filtered through a Pasteur filter; and the difference in the two estimations will represent the amount of suspended matter retained by the filter.

The various forms of animal and vegetable life (exclusive of bacteria), and of inanimate organic and inorganic matter, are best sought after by commencing with the ½-inch power, and next passing on to the ¼-inch power.

The presence of living organisms in abundance, bearing as they generally do a ratio to their food-supply, will be often sufficient in itself to condemn the water as containing a considerable amount of organic (mainly vegetable) pollution. The fact that animal and vegetable life have powers of purifying the water is beside the question; their very presence denotes impurity, and with the attainment of purity they mostly disappear.

The higher and macroscopical types of animal life, such as water-fleas and other crustacea, broadly speaking, denote less danger than the lower and more minute forms (bacteria, amœbæ, infusoria). The former are generally associated with suspended matter in waters that are not likely to be used for drinking purposes on account of their contamination being obvious to the senses, while the latter are often found to be associated with dissolved organic matter in waters which may possess excellent physical characters.

More especially do large numbers of bacteria and the presence of fungi, infusorians, and anguillulæ, suggest harmful pollution. But the most suspicious elements which may be detected by a microscopical examination of suspended or deposited matter are those which point directly to sewage contamination, and those which point indirectly to human contamination. The latter will be found in hair, wool, cotton and linen fibres, epithelial scales, etc. The former include objects which can rarely gain access to water save in actual sewage, such as: (1) Substances which from their indigestibility commonly leave the body in the fæces; (2) substances which may do so when digestion is interfered with; and (3) eggs, etc., of animal parasites which infest the human gastro-intestinal tract. Under the first heading would be embraced such substances as—Various connective-tissue elements, fat globules and crystals, muscle fibres, starch cells, etc. Under

the second heading, shreds of mucous membrane, epithelial cells, gall-stones, particles of various kinds of foods in a semi-digested state, etc. The third heading would include *T. solium*, *T. mediocanellata*, *Bothriocephalus latus* (either as eggs or segments), *Ascaris lumbricoides*, *Oxyuris vermicularis*, *Tricocephalus dispar* (ova or mature forms), *Paramœcium coli*. It must not be thought that this direct evidence of human contamination is obtainable except on rare occasions.

Matter which may be found by a Microscopical Examination of Water.

1. Inanimate.

(*a*) *Mineral.*—This may be examined by the ¼-inch power, and then chemical tests applied under the cover-glasses.

Sand and flint particles generally have a sharp and angular

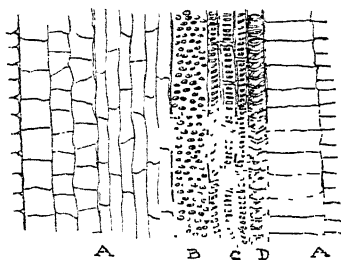



FIG. 12.—VEGETABLE TISSUE.
A, Vegetable parenchyma; B, a pitted vessel; C, a scaliform vessel; D, a spiral vessel.

outline, though often somewhat rounded from rolling and attrition; a drop of hydrochloric acid let to run under the cover-glass has no effect upon them.

In clay and marl (silicate of alumina) the particles are amorphous and very minute, and unaffected by hydrochloric acid.

Chalk particles are likewise amorphous, mostly somewhat larger than those of clay and marl, and generally rounded in outline. A drop of hydrochloric acid causes them to disappear with effervescence.

Iron peroxide forms a yellowish-brown amorphous débris, soluble (like the chalk particles) in hydrochloric acid, and blued

when a drop of HCl and of a solution of ferrocyanide of potassium are allowed to run under the cover-glass.

Mica forms thin, fine, scale-like films of very irregular outline, and insoluble in hydrochloric acid.

(*b*) *Vegetable.*—Parenchyma; the dotted ducts and spiral vessels or spiral fibres; pieces of the cuticle with the vegetable "hairs" still adhering; pollen.

Woody fibres; fragments of leaves, etc.; starch cells; macerated paper; linen and cotton fibres; dark particles of soot.

Vegetable matter often appears as dark flattened structureless particles, or frequently only as débris, when the classification of the deposit is attended with great difficulty; if, however, any spiral vessels, or fibres, or dotted ducts can be distinguished, these will always point to the probable nature of any obscure débris, etc., with which they are associated.

(*c*) *Animal.*—Hairs; feathers; down; wool or silk fibres.

Striped muscular fibres; fat globules and crystals; connective tissue; epithelial scales; shreds of mucous membrane.

Scales and wings, legs, etc., of insects.

Reddish-brown globular masses are sometimes found in association with grosser sewage pollution. Their nature is somewhat obscure.

The following particulars will serve for the microscopic identification of textile fibres (*vide* Plate I.).

Linen.—Cylindrical jointed fibres, with minute branching filaments at intervals.

Cotton.—Flattened twisted fibres, with no joints or nodes and no branching filaments or transverse markings. The apex of the fibre is blunt.

Wool.—Rounded fibres, with fine cross-markings and indentations on the border at the site of the cross-markings. A central longitudinal canal exists, but this is generally obliterated. The fibres stain yellow with a saturated aqueous solution of picric acid, warmed and then allowed to cool—not so those of linen.

Silk.—Long cylindrical fibres with a well-defined central canal, but no cross-markings or indentations. The fibres dissolve rapidly in concentrated H_2SO_4—not so those of wool.

Hemp.—Fibres faintly striated, with central canal, and transverse and oblique lines cross the fibres. (These are rendered more distinct by chloral hydrate.) A transverse section of fibres is irregularly rounded. The ends of the fibres are usually blunt.

Flax.—Closely similar to hemp, but the ends of the fibres taper to fine points. The fibres are polygonal in transverse section.

2. Animate.

Vegetable.—Minute forms of vegetable life mostly belong to the class of cryptogamous (*i.e.*, non-flowering) plants, and contain chlorophyll. They may be divided into—

1. *Small and microscopic fungi*, which represent some of the lowest forms of vegetable growths. These may be present as spores, sporangia, or mycelia.

Both *Bacterium termo* and *Sarcina ventriculi* present familiar instances of these forms.

Beggiatoa alba has been badly named "the sewage fungus," but any water containing a high amount of sulphuretted hydrogen or sulphate is capable of supporting this fungus, quite independently of the source from which such sulphur compounds are derived, and certain other growths are found even more frequently in association with sewage pollution. In *Beggiatoa alba* the sheathless cylindrical filaments contain roundish particles of sulphur, which are highly refractile to light.

Leptothrix presents under the microscope a similar appearance to that of *Beggiatoa alba*, but the cylindrical cells, connected in threads and surrounded by a sheath, exhibit no sulphur granules. The empty sheaths may form large brownish deposits in water containing iron. *Leptomitus lacteus* forms soft white or dirty tufts attached to stones or channels. In the absence of oxygen the growth darkens and putrefies. Under the microscope long branching filaments, constricted at regular intervals and bearing zoospores on the terminal segments, are seen. *Sphærotilus natans* is a similar growth, presenting under the microscope chains of long undivided filaments. *Cladothrix* may be found to be abundant in iron waters; the threads are composed of rod-shaped cells surrounded by a thin sheaf.

An aquatic plant known as *crenothrix* gives much trouble at times, because of its tendency to develop in the water-mains and to clog water-pipes. Under the microscope this plant presents cylindrical filaments transversely divided into cells, and these filaments are surrounded by a gelatinous sheath which is coloured by a deposit of ferric oxide. The cells may, by division and production of viscous matter, escape and form zooglœa. A

drop of dilute hydrochloric acid let under the cover-slip dissolves up the iron, and enables the plant structure to be more clearly defined. Iron is requisite for its growth, and it is absent from waters which are but slightly ferruginous. It is often discovered in mains quite unexpectedly, and its long rusty filaments have been sometimes taken for horse-manure, with a consequent poor opinion of the character of the water-supply. Removal of the iron by oxidation and filtration is the best guarantee against the occurrence of this growth.

Mucor, *Aspergillus*, and *Penicillium* are moulds which may often be found in stagnant water. These are illustrated on pp. 261, 262, and 279.

2. *Numerous forms of algæ*, ranging in size from those visible only at high microscopic powers to those visible with the naked eye. Of these there are many families: The volvocineæ, of which *volvox* is the type, include the lowest vegetable forms of minute organisms; the oscillatoria exhibit a pendulum-like motion; the confervaceæ are very numerous.

Volvox globator forms a green colony of cells. The colony is spherical, and the individual cells live in the wall of the sphere, each having two flagella and a necleus. The ball, or hollow colony, continually rotates by means of the flagella of each individual. This organism gives rise to a fishy taste and odour in water.

Protococcus pluvialis is an interesting instance of an algoid plant which can live in the atmosphere, and which may be found in rain-water. *Raphidium* and *Scenedesmus* are not uncommon.

Clathrocystis and *Microcystis* are widely distributed in surface-waters.

Of diatoms, *Asterionella* and *Navicula* are familiar types.

The algæ, when in considerable quantities, may furnish a dark green, repulsive appearance to the water, and may give rise to diarrhœa; when they die and decay the water acquires an offensive taste and odour.

In winter comparatively few of such growths are found. In spring various diatoms appear; these will disappear in a few weeks, and in their place will come the green algæ; in the fall these will disappear, and the diatoms develop again—in turn to disappear with the onset of winter (Whipple). They (*tabellaria*, *asterionella*, etc.) occasionally grow in large numbers on the surface of stagnant water, or even on filter-beds.

Animal.—1. Protozoa. (*a*) Rhizopoda. *Amœba* will be recognized by its characteristic amœboid movement. *Amœbæ coli* are found in the mucus of the discharges of persons suffering from dysentery. *Actinophrys*, the body of which is surrounded by stiff radiating pseudopodia, is another common and familiar form; and *polyps* shows a very low type of structure. In *Spongilla fluviatilis* (the fresh-water sponge) the animal substance is spread over a network of spicules; it grows in green masses.

Difflugia has a coating formed by sand which it has taken into its body, and with which it makes a globular shell inside of which is the soft protoplasm of the body substance; this flows out at the mouth of the shell and forms anastomosing threads, which, acting as a net, catch food. *Cercomonas* and *Euglena viridis* are also common. *Euglena* is actively motile and green from chlorophyll.

(*b*) Infusoria. *Paramœcium*, *vorticella*, and *coleps* are all common types.

Stentor is among the largest of this class, and is so named from the trumpet-like shape of the body. It is covered with small cilia, and possesses long cilia in front.

Coleps is also covered with cilia, and has an opening surrounded by short spines at both ends.

Paramœcium is flattened and covered with cilia. *Paramœcium coli* are sometimes associated with diarrhœal discharges in human beings, and *Cercomonas intestinalis* (a protozoan) may be present in the mucous discharges of children. *Paranema* is one of the simplest of the infusoria; it possesses one flagellum. *Vorticella* possesses a long contractile stalk. The body has a lid, and both the lid and the opening into the gullet are ciliated.

2. Cœlenterata.—*Hydra* is a common type of this sub-kingdom.

3. Annulosa.—This sub-kingdom embraces—

(*a*) Crustacea, including the amphipoda,* isopoda,† and branchiopoda.‡

* The amphipoda are sessile-eyed malacostracans. Their bodies are compressed laterally, the eyes are immobile, and the feet are directed partly forwards and partly backwards.

† The isopoda possess sessile eyes and a depressed body, and the feet are of equal size and move in the same direction.

‡ The branchiopoda are so called because their branchiæ or gills are situated on the feet. The head is not distinct from the thorax, which is much reduced in size.

Cyclops quadricornis, Gammarus pulex, and *Daphnia pulex* are familiar types of this class. In the latter the antennæ act as oars and propel the little animal through the water by a series of short springs or jerks; they assume a red colour in summer, and when in swarms they give a bloody tinge to the water.

(*b*) Arachnida, including the microscopic tardigrada or "water-bears."

(*c*) Insecta. Either in the larval, pupal, or adult forms.

4. Annuloida.—This sub-kingdom embraces the scolecida, and includes turbellaria, rotifera (or wheel-animalcules), tæniadea, næmatoidea, anguillulæ (water-worms).

Rotifer vulgaris.—Gives a red or green colour to gutter-water. It is often called the "wheel" animalcule on account of its circular, oval disc, which is fringed with cilia; it is motile, and by its movements conveys an appearance of rotation; the cilia serve to propel the animal and to set up food currents.

Small water-leeches (*Hirudinidæ*) found in fresh-water and on wet grass may be distinguished by their two suckers, one at either extremity; the mouth, armed with three teeth, is set in the middle of the anterior sucker.

5. Mollusca.—Including polyzoa, siphonida, etc.

The various **human parasites** which may be conveyed through the medium of water are—

The segments and eggs of tape-worms (*Tænia solium, T. mediocanellata, T. echinococcus,* and *Bothriocephalus latus*); the Guinea-worm (*Dracunculus medinensis*); the round-worm (*Ascaris lumbricoides*); the threadworm (*Oxyuris vermicularis*); *Bilharzia hæmatobia; Ankylostomum duodenale; Tricocephalus dispar* (the whip-worm); *Filaria sanguinis hominis;* the filarial stage of *Distoma hepaticum* (the liver-fluke of sheep).

Several of these may be found in either embryonic or adult stages of development.

The ova of *bothriocephalus* are developed in fishes, and man can only be infected by eating the latter. The larva of *Ankylostomum duodenale* is developed from ova in foul waters, and man may become infected by handling and drinking such water. The smooth oval egg of *Ankylostomum duodenale* possesses a segmented yoke when it leaves the female. After being discharged in fæces, under suitable conditions of moisture and temperature an embryo forms, which after a few days passes a sluggish exist-

ence usually in damp mud or earth. It may thus live for weeks or months, and if it gains access to the small intestine of the human being the worm reaches sexual maturity in about a month. The adult worm is a small nematode with a mouth furnished with four strong projecting hooks and two conical teeth, and the tail of the male has a large umbrella-like expanded bursa from which two long thin spicules project.

Man is very rarely infected by the larvæ of *Distoma hepaticum*. The ova develop in water into ciliated embryos, and these undergo in small water-snails (*Limnæus truncatulus*) a further development to larvæ; these ultimately change into little organisms (*cercaria*) resembling tadpoles, which either remain encysted in water-snails or leave them and become attached by their suckers to grass.

Bilharzia hæmatobia.—The male is a grey flattened trematode worm, ½ inch in length, which inhabits the portal, splenic,

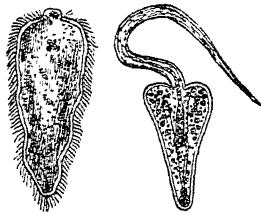



FIG. 13.—CILIATED EMBRYO AND CERCARIA FORM OF DISTOMA HEPATICUM (MAGNIFIED).

mesenteric veins, and the inferior vena cava of man and of some species of apes, especially in Africa and India; posteriorly the sides of the parasite curve towards each other and meet to form a channel (the gynecophoric canal), in which part of the long slender female (¾ inch in length) lies during fecundation. The spindle-shaped ova possess a beak, which generally projects from one end, but sometimes laterally; these ova may be hatched before the parasite leaves the tissues of the original host, but the embryos are not born until afterwards. If the ova find their way into water, their walls swell up and rupture and the minute embryos escape, armed with cilia which serve to project them through the water. Apparently the embryo becomes attached to some fresh-water mollusc (or possibly fish), and develops into a *cercaria* form. Probably man is not infected by drinking-water, but by protracted immersion in contaminated water, when

the parasite enters the urethra or anus. The parasite is incapable of reproducing itself within the human body.

The embryo of *Filaria medinensis*, or the Guinea-worm, is also aquatic in habit—indeed, its first stages of development occur in a fresh-water crustacean. From these facts the inference that the parasite is transferred to man by drinking-water is justifiable.

Of the *Filaria sanguinis*, the embryo of one of these (*Filaria nocturna*) has been traced through the body of the mosquito, and so into water, by which it may enter the human body—although it is usually inoculated directly by the bite of the insect.

Tricocephalus dispar is also transmitted by water, for the egg develops only in water or upon some very damp medium, and the liberated embryo may thus find its way and attach itself to the mucous membrane of the cæcum.

There are other human parasites which infect their host frequently through the medium of drinking-water, although their life-histories do not even include a temporary residence in water. Thus the ova of *Ascaris lumbricoides* and of *Tænia echinococcus* are often washed into water or blown into it as dust. The ova of the female ascaris are discharged with the fæces of the host, when, but not before, they are capable of furnishing embryos; these probably have an independent existence (possibly in water or some intermediate host, such as worms or insects) before again entering the human body and completing their development.

It may be stated that, as a general rule, with one or two exceptions—such as *Oxyuris vermicularis*, *Trichina spiralis*, and the *tape-worms*—contaminated water is the principal means by which the entozoa of man pass into his system. The ova of *O. vermicularis*, unlike those of *A. lumbricoides*, contain embryos prior to their discharge, but probably these are incapable of further development until they have been passed with the fæces, when they may reinfect the same individual or others occupying the same bed, etc.; or may pass into water, or get deposited upon vegetables and fruit, and thus get ingested.

The animal parasites of the lower animals also supply many instances of transmission by water.

CHAPTER IX

ORGANIC MATTER IN WATER

ORGANIC pollution may be of *animal* and *vegetable* matter; and since the danger of these two forms of organic contamination differs very materially (animal matter being far more dangerous than vegetable), it is important to learn the nature as well as the amount of any organic matter which is fouling water.

Organic matter, as is well known, becomes, under suitable conditions of temperature, air, and moisture, resolved into simpler parts by fermentation, putrefaction, and slow oxidation. As the ultimate result of these processes, the carbon appears as carbonic acid, the hydrogen as water, and the nitrogen as ammonia, nitric and nitrous acids. When putrefaction sets in, odorous gases are evolved, which mostly consist of compounds of sulphur.

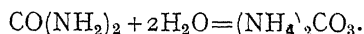
The effort to estimate the organic matter from the amount of oxygen of which it will deprive the permanganate of potassium was practised almost universally for a long period, and it remains as an auxiliary test for organic matter to this day. The facts that potassium permanganate in solution is so very unstable, that other substances in the water—apart from organic matter—are capable of reducing it, that it will part with its oxygen readily to the less dangerous (*i.e.*, vegetable) as well as to the more dangerous (*i.e.*, animal) pollution, and that the oxidizable organic matter bears an unknown and inconstant ratio to the *total* organic matter, all conduced to some dissatisfaction with the test, and endeavours have been made to find others which are of greater service.

E. Frankland devised a most ingenious process to meet the want, but it is quite unsuited to the bulk of health officers, and there is scope for some error of experiment to creep in even with practised hands. In this process a measured volume of water

is evaporated to a solid residue, and this is collected in a hard glass combustion tube, mixed with oxide of copper, and burnt in a furnace. The oxide of copper parts with its oxygen to the organic matter, which is completely destroyed, and the carbonic acid, nitric oxide, and nitrogen which result are collected, measured, and expressed in terms of "organic carbon" and "organic nitrogen."

A method superior in the facility of its execution, and equally as valuable for the purpose at issue, is that known as "the Wanklyn, Chapman and Hall Process." By it an endeavour is made, after computing the amount of "free and saline" ammonia *originally present* in the water, to estimate the amount of nitrogenous organic matter present from the amount of ammonia which can be derived from the breaking up of such matter by strongly alkaline permanganate of potassium at the boiling temperature. The organic matter which gains access to water is largely nitrogenous, and a very delicate indication of its presence and amount may be obtained from the nitrogen which it furnishes.

Great importance is also attached in this process to the amount of the "free and saline" ammonia originally in the water, for it is, generally speaking, a product of recent animal contamination. It will be recalled that one of the chief nitrogenous substances in sewage is urea; and this urea, by the action of the *Micrococcus ureæ*, is rapidly converted into saline ammonia, thus:

$$CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3.$$

It is obvious that no chemical process can determine as to whether the organic matter is living or dead, or whether in the former case it is harmful or not; but while considerable quantities of the germs of disease cannot by themselves appreciably affect the amount of "albuminoid ammonia," since they always gain access to water along with other organic matter, this latter often furnishes by chemical analysis the evidence of danger.

CHAPTER X

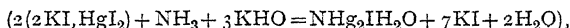
WANKLYN'S PROCESS

Special Reagents required :

1. A standard solution of chloride of ammonium, made to the strength that 1 c.c. contains 0·01 milligramme of ammonia.

The solution is made by dissolving 3·14 grammes of pure chloride of ammonium in a litre of distilled ammonia-free water; if some of this is diluted a hundredfold with distilled ammonia-free water, it is of the required strength.

2. Nessler's reagent. This consists of a saturated solution of the per-iodide of mercury in distilled ammonia-free water, the whole being rendered strongly alkaline with caustic potash. When this reagent is applied to a solution containing ammonia, it imparts a colour varying from a faint yellow to a reddish-brown, or even a precipitate, according to the amount of ammonia present. This reaction, which is due to the formation of ammonio-mercuric iodide,

$$(2(2KI,HgI_2)+NH_3+3KHO=NHg_2IH_2O+7KI+2H_2O),$$

is not shared by *organic matter as such*.

The solution of the reagent should have an extremely faint yellow colour, which indicates that it is saturated with the periodide of mercury, and is therefore " sensitive "; should it be colourless and non-sensitive, this can be corrected by the addition of a drop or two of a saturated solution of corrosive sublimate.

Any precipitate of mercuric iodide which settles should not be disturbed when the reagent is being used.

The following has been found the best method of preparing Nessler's reagent: Dissolve 13 grammes of corrosive sublimate in about 250 c.c. of water, and 35 grammes of iodide of potassium in another 250 c.c. of water—by boiling; mix the two hot solutions, when a precipitate of the red per-iodide of mercury forms, which redissolves in the excess of iodide of potassium present; add a cold saturated solution of corrosive sublimate [illegible] of red periodide just begins to remain permanently; raise to the boiling-point, and the precipitate will possibly be dissolved; allow the solution to become cold, or cool under the tap in a suitable vessel, and decant from any precipitate; then dissolve 120 grammes of caustic potash in about 400 c.c. of water, and cool this solution; mix the two cold solutions, and make up to 1 litre of Nessler's reagent with water. Ammonia-free water must be used throughout.

The reagent should be kept in a tight-fitting glass-stoppered store bottle, and small quantities emptied out into a smaller one for use from time to time. It is most sensitive after it has been kept for some time.

3. A strongly alkaline solution of the permanganate of potassium, which should always be boiled for a few minutes prior to use, in order to get rid of any traces of ammonia.

The amounts recommended to be used in making up the stock solution are—

Caustic potash, 200 grammes.
Permanganate of potassium, 8 grammes.
Ammonia-free distilled water, to 1 litre.

Ammonia-free distilled water may be made by distilling tap-water, after first fixing the ammonia present by the addition of a drop or two of dilute sulphuric acid; the distillate from the bulk of a litre of the water can then be collected as "ammonia-free." As the previously ammonia-free water is very liable to take up traces of ammonia, it should always be carefully tested prior to use, and any trace of ammonia present must be distilled off.

Special Apparatus required:

Six Nessler glasses. These are narrow cylinders, each marked off at a point which indicates the level to which 50 c.c. of water will stand in them; they should be made of thin colourless glass, and of precisely similar diameter.

Condensing apparatus, as shown in Fig. 14. The small tube perforating the stopper of the boiling-flask is seen to be surrounded in the greater part of its length by a larger tube. A constant circulation of cold water in the space between these tubes causes a condensation of the steam which arises from the boiling water, this distillate being received into Nessler glasses.

A white porcelain slab, 6 inches by 4 inches.
A mounted graduated burette.
A 2 c.c. pipette.

The Process.

The amount of "free and saline ammonia" is first estimated—*i.e.*, that ammonia which exists in solution in the water, or in combination with acids (carbonic, nitric, etc.), or in some other easily decomposable form.

The Nessler reagent will create the *faintest possible* evidence of a yellow colour in 50 c.c. of the sample when this contains only a very small amount of "free ammonia." It is well to make it a practice to test the water in this way before commencing Wanklyn's process, in order to know whether the sample contains little or much "free and saline" ammonia. If more than a faint yellow tint forms, the water should be diluted, as it may otherwise be difficult to get the large amount of ammonia over and to match it. For instance, in the case of extremely foul waters, the degree of colour due to the ammonia in the first 50 c.c. of distillate is too intense to be matched by the standard solution, for in many cases a copious precipitate

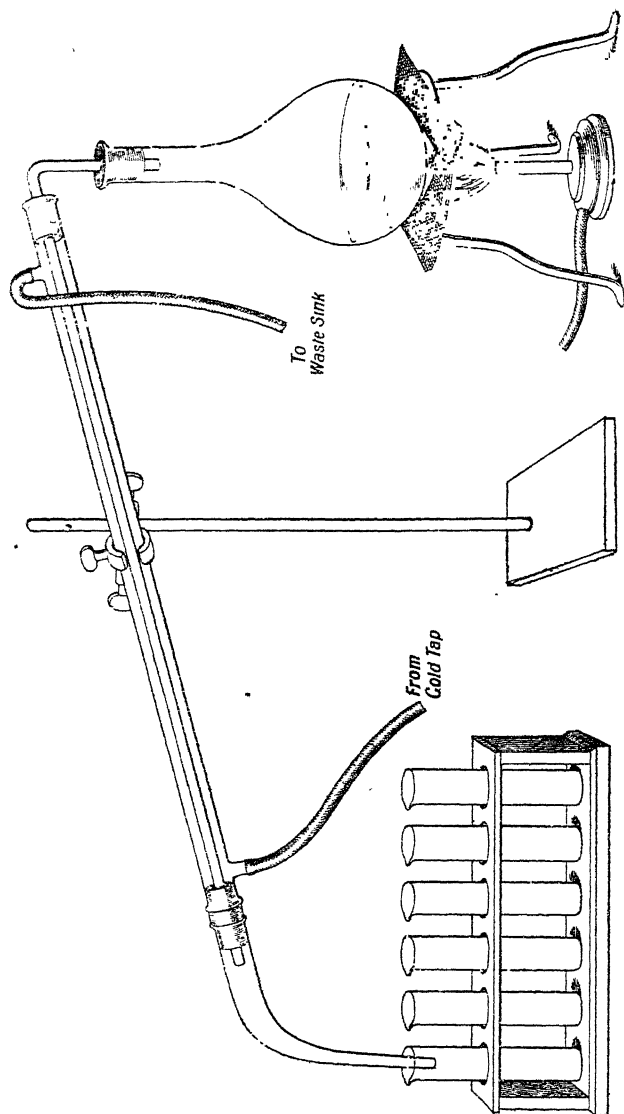



FIG. 14.—APPARATUS FOR WANKLYN'S PROCESS.

appears, and it is impossible to make a comparison. In such cases, smaller quantities of the original water should be diluted with an equal bulk, and sometimes even with five or ten times its amount, according to the depth of yellow colour obtained, of distilled ammonia-free water, prior to distillation.

1. The condenser is liable to become contaminated with ammonia. Therefore first distil some clean water through the apparatus until the distillate gives no colour with Nessler's reagent.

2. Five hundred c.c. (*i.e.*, half a litre) of the water are placed within a boiling-flask.

If the water is acid or even neutral, a little pure anhydrous sodium carbonate should be added so as to insure alkalinity. The motive for this is to enable the free ammonia to come away readily, since any acidity would exert a fixing influence upon it; it also decomposes ammonium sulphate.

3. The boiling-flask is then tightly connected to the condenser, so that no uncondensed vapour can escape at this point. The Bunsen burner is next lighted, the flame applied to the flask, and *rapid* boiling is encouraged.

4. The water-tap is turned to such an extent that the water, after circulating in the outer tube of the condenser, flows in a small stream to the waste-sink.

5. A Nessler glass is placed so as to catch the distillate, and when sufficient of this is collected so as to reach up to the level of the 50 c.c. mark, a second glass is substituted, and then a third.

6. When three Nessler glasses are thus filled up to their 50 c.c. marks with distillate, a fourth is placed to catch more of the distillate, while 2 c.c. of Nessler's reagent are added to each of the three glasses. If these glasses be placed upon a white porcelain slab from left to right in the order in which they received the distillate, the yellow colour furnished in each of them by the reagent will show a decrease in amount from left to right, since the first 50 c.c. collected will contain the most "free and saline ammonia," and the third the least.

7. The gas may be turned out and the distillation stopped if there is no colour in the third Nessler glass, or if it be *extremely* faint, since all the "free and saline ammonia" will then have come over. If, however, the colour is distinct in the third Nessler glass of distillate, a fourth must be collected and tested

with 2 c.c. of the reagent, and even a fifth may be occasionally necessary. It is, of course, imperative that *all* the "free and saline ammonia" in the original 500 c.c. of water shall be removed, and it is seldom in a drinking-water that 150 c.c. of distillate does not contain the whole of this.

8. The amount of ammonia must be determined by matching the colour in each glass. To make this match it is necessary to take another Nessler glass, and to deliver into it by a burette the amount of ammonium chloride standard solution that is judged necessary to effect the match; the cylinder is then filled up to the 50 c.c. mark with distilled ammonia-free water, and then 2 c.c. of Nessler's reagent are added. If the match is not correct, then a fresh comparison must be made with more or less of the standard solution, as the case may be. A very little experience will enable the operator to effect this matching with great rapidity.

Notes.—It is pointed out by Wanklyn that it is not necessary to match each glass separately, since three-quarters of the total amount of "free and saline ammonia" is contained in the first glass of distillate. Occasionally, however, there is a faint discrepancy between the amount thus calculated and that obtained by matching each glass; this may be due to differences in the degree of alkalinity of waters and in the rate of boiling. On this account, and for the reason that the statement does not hold true with very foul waters, it is preferable in every case to estimate the amount of ammonia in each glass.

In every case when, after stirring with a clean glass rod, the colour in the comparison cylinder is found to approach that in the distillate, the operator should cover up the cylinder and wait about three minutes before adding more standard solution, since the colour deepens a little upon standing.

The presence and degree of coloration must always be judged by looking down through the depth of the water on to a white slab, and care must be taken that the bottoms of the glasses and the upper surface of the slab are perfectly dry, as a thin layer of intervening water diminishes materially the depth of colour, and leads to error in matching.

9. Having thus effected a colour match by placing the two glasses side by side upon the white slab under exactly the same conditions of light access, the amount of ammonium chloride solution which has been used to effect this is noted, and the

ammonia which this is equivalent to will be the amount of the "free and saline ammonia" in the glass of distillate.

Example.—One hundred and fifty c.c. of distillate were collected, and the last 50 c.c. are found to contain no trace of ammonia. The whole of the "free and saline ammonia" in the 500 c.c. of water was therefore collected in two Nessler glasses.

It was necessary to add 3 c.c. of the standard solution of ammonium chloride to the comparison test-glass in order to match the colour in the glass containing the first 50 c.c. of distillate, and 1 c.c. of the standard solution was required to match the colour in the second 50 c.c. of distillate.

The total amount, then, of "free and saline ammonia" in the 500 c.c. of water corresponds to the ammonia present in 4 c.c. of the standard solution of ammonium chloride.

But 1 c.c. of this standard solution contains 0·01 milligramme of ammonia. ∴ 4 c.c. contain 0·04 milligramme of ammonia.

∴ there is 0·04 milligramme of "free and saline ammonia" in the 500 c.c. of water (or 500,000 milligrammes).

∴ there is 0·008 milligramme of "free and saline ammonia" in 100 c.c. (100,000 milligrammes) of water, or 0·008 part per 100,000.

10. *The next step* in the process is to continue the distillation more slowly after adding 50 c.c. of the recently boiled alkaline solution of permanganate of potassium to the boiling-flask; to collect the distillate in three Nessler glasses; and to repeat the process of "Nesslerizing" precisely as before. The ammonia now obtained is called "*albuminoid ammonia*," since it is derived from the breaking up of albuminoid and other nitrogenous organic matter by means of the alkaline permanganate. It is important to remember that this albuminoid ammonia comes over more slowly and much less evenly (the second Nessler glass sometimes containing almost as much as the first), so that the first 50 c.c. of distillate must never be taken to contain three-quarters of the total "albuminoid ammonia."

Example.—It was necessary to distil over 200 c.c. in four Nessler glasses before all the ammonia had come over. The fourth glass of distillate had colour equal to that furnished by 0·2 c.c. of the standard solution, the third to 0·8 c.c., the second to 2 c.c., and the first to 2·5 c.c.

∴ $(0{\cdot}2 + 0{\cdot}8 + 2 + 2{\cdot}5) = 5{\cdot}5$ c.c. of the standard solution were

required to match the colour furnished by the "albuminoid ammonia" in 500 c.c. of water.

But each c.c. of the standard solution = 0·01 milligramme of NH_3. ∴ 5·5. c.c.=0·055 milligramme of NH_3.

∴ there is 0·055 milligramme of NH_3 ("albuminoid") in 500,000 milligrammes of water, or 0·011 milligramme in 100,000 of water.

Conclusions to be Drawn from the Amount Estimated.—In the case of contamination with animal matter the "free" ammonia exceeds the "albuminoid"; while vegetable matter furnishes "albuminoid" ammonia and practically no "free." Therefore much "albuminoid" along with a very small amount of "free" ammonia indicates vegetable contamination, and this indication gains further support if there is no excess of chlorides and of nitrates. Relatively high "free" ammonia, along with "albuminoid" ammonia above 0·005, and excess of chlorine (and often of oxidized nitrogen) will denote recent animal pollution.

If the "albuminoid" ammonia exceeds 0·005 part per 100,000, the "free" should be below this amount; but if the albuminoid ammonia is much below this, then a high figure of "free" ammonia is probably due to a reduction of nitrates, and not to recent animal contamination. Conversely, if there is practically no "free" ammonia—*i.e.*, 0·002 or less—then the "albuminoid" ammonia may be allowed to exceed 0·01, as it is evident that the organic matter present is purely vegetable.

It may be said that if the free ammonia in upland surface-waters exceeds 0·002, a suspicion of animal contamination is warranted.

"Free" ammonia, accompanied by practically no "albuminoid," is found in the following circumstances:

(*a*) The water has been in contact with a stratum containing a reducing agent (greensand contains a reducing salt of iron) which has decomposed the oxidized nitrogen originally present in the water; or metal pipes, cement, etc., with which a well-water has come in contact may effect this reduction to a less extent.

(*b*) Other waters containing iron frequently possess a marked amount of ammonia derived from the reduction of nitrates.

(*c*) The water has percolated a deposit in which some ammonia salt is present.

(*d*) The sample is rain-water, collected in town districts, in which ammonia may exist in considerable quantities.

Other steps of the analysis will serve to indicate the source of "free ammonia"; and where it is not derived from organic pollution the "albuminoid ammonia" will always be very low indeed.

If, in spite of the previous dilution, the ammonia in the first Nessler glass of distillate still furnishes too deep a colour to admit of a satisfactory match, the whole of the distillates containing free ammonia may be mixed together, and the ammonia in 50 c.c. of the light-coloured *mixture* Nesslerized and estimated; and from the amount found in this measured part the amount in the whole distillate may be calculated.

Sometimes while extracting the "albuminoid ammonia" the contents of the boiling-flask boil too violently, and "bumping" ensues; to obviate this a gentle shaking of the flask will often suffice, but in default a few fragments of freshly ignited pumice-stone afford an excellent remedy. The foulest waters and those containing much saline matter are most apt to bump, and it is highly important to prevent this, since uncondensed vapour thereby escapes at the distal end of the tube, and sometimes some of the water is shot over from the boiling-flask, both of which occurrences obviously vitiate the results. When some of the water to which the alkaline permanganate has been added thus spurts over into the Nessler glass placed to collect the distillate, it is of course impossible to "Nesslerize," since the distillate has a pink colour. There is no alternative then but to pour back the distillate into the flask and renew the distillation.

When the "albuminoid ammonia" comes over so slowly (as in some peaty waters) that almost all the water in the retort threatens to be used up, 200 c.c. of ammonia-free water may be added to the flask and the distillation continued. In those rare cases where "the free ammonia" continues to come over in small quantities, it is a good plan to adopt the measure (Rich) of starting the process afresh, "Nesslerizing" the first 50 c.c., and then returning the rest of the distillate to the flask, and redistilling it before "Nesslerizing."

Strange to say, though urea is decomposed by the boiling with

the alkaline permanganate, its decomposition does not yield any ammonia, and this at first sight would seem a grave defect in the process. When, however, it is considered that this is probably the only nitrogenous contamination of animal origin with which a water is liable to be polluted which does not, in the circumstances, yield ammonia, and that urea in urine naturally becomes very rapidly changed into ammonium carbonate, and as such is detected in the saline ammonia, the matter is not one of importance.

By Wanklyn's process only about one-half of the nitrogen in organic combination is liberated as "albuminoid ammonia"; but it is not necessary that in the process the *total* nitrogen contained in organic matter should be evolved as ammonia, so long as that which *is* evolved gives an index which bears a fairly fixed and constant ratio to the total amount; so that from this index an empirical standard of purity can be formed. The process efficiently meets this requirement.

When but little water remains in the boiling-flask, the flame must be lowered, as the naked flame must not be allowed to play upon the glass above the water-level.

The presence of considerable sulphuretted hydrogen in water interferes with Nesslerization; this must therefore first be remedied before the free and saline ammonia are distilled over and estimated in the following manner: The ammonia should be fixed with 10 c.c. of normal sulphuric acid; then, if 100 c.c. of the water are distilled over, this amount of distillate will contain all the sulphuretted hydrogen. The water remaining in the boiling-flask is then neutralized with 10 c.c. of normal sodic hydrate, when Wanklyn's process can be performed.

When it is found necessary so to deal with sulphuretted hydrogen, a blank experiment should be performed, by which any ammonia found in 500 c.c. of ammonia-free distilled water containing 10 c.c. of normal sulphuric acid and 10 c.c. of normal sodic hydrate is distilled over and estimated, and this is deducted in arriving at the figure of the free and saline ammonia in the sample.

CHAPTER XI

THE OXIDIZABLE ORGANIC MATTER—E. FRANKLAND'S PROCESS

IN the presence of organic matter the permanganate of potassium, under favourable conditions, will part with oxygen until all the permanganate has become reduced to hydrated manganese dioxide, as indicated by the loss of the original pink colour.

While a certain proportion of the organic matter present in water is always oxidizable by the permanganate of potassium, this varies with the nature of the organic pollution, and it therefore bears no constant ratio to the total quantity of such pollution present. Some forms of animal matter reduce less permanganate than others, and comparatively harmless peaty waters may absorb much more oxygen than waters dangerously polluted with animal matter.

Despite these drawbacks, and the fact that in Wanklyn's process we possess the means of making a far closer estimation of organic matter, the test under consideration frequently furnishes corroborative evidence of value, but it is most serviceable as a means of gauging the comparative purity of a series of waters, or of the same water from time to time.

A two-hours' exposure of the water to the permanganate is quite short enough for the test to be of much value, since it is chiefly the *putrescent* or very easily reducible organic matter which is oxidized in the first half-hour. There is little or no advantage, however, in making the test extend to four hours. It must be clearly understood that even at the end of four hours the oxidation of the more stable organic matter by acid permanganate would be incomplete; and so the two-hours test is generally adopted for the purpose of obtaining a standard or figure for comparison.

The conduction of the process at a precise temperature has

been proved by experiment to be an important factor, for the amount of oxygen taken from the permanganate varies considerably at different temperatures.

If the water is bottled long before analysis, the quantity of oxygen absorbed frequently increases; this is due to the fact that the organic matter is slowly passing into less stable forms (and is therefore less resistant to the permanganate), and rarely it may also result from a reduction of nitrates by organic matter (bacteria, etc.) to nitrites.

TIDY'S MODIFICATION OF THE FORCHAMMER PROCESS.

Special Reagents required:

1. A standard solution of the permanganate of potassium, 10 c.c. of which contain 1 milligramme of available oxygen, made by dissolving 0·395 gramme of the pure salt in a litre of distilled water which has been faintly tinged with permanganate solution to oxidize any impurities present. The solution is unstable, and must be frequently renewed.
2. A freshly prepared solution of potassium iodide, made by dissolving one part of the pure salt in ten of distilled water.
3. Dilute sulphuric acid (1 in 3); a solution of the permanganate of potassium is dropped in until a faint pink tint remains after four hours at a temperature of 27° C.
4. A solution of sodium thiosulphate, made by dissolving 1 gramme of the crystallized salt in a litre of distilled water.
5. A freshly prepared solution of starch, made by adding 0·5 gramme of well-washed starch to 200 c.c. of cold distilled water, and briskly boiling for five minutes; then let settle and decant the almost clear supernatant liquid.

Special Apparatus required:

Two thin glass-stoppered bottles or flasks of only a little more than 100 c.c. capacity.

Two thermometers graduated on the Centigrade scale.

Graduated burettes; glass stirring rods; white porcelain slabs.

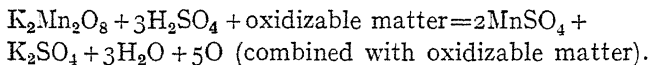
The Process.

1. To 100 c.c. of the water in one of the thin glass flasks add 10 c.c. of the dilute acid; then add 10 c.c. of the standard solution of permanganate, and insert the stopper.

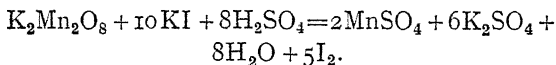
2. The solution of thiosulphate is unstable, and it is therefore advisable to always include a control test as follows: 100 c.c. of cold *recently boiled distilled* water are treated in precisely the same manner.

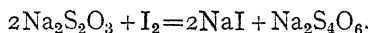
3. Place both flasks in a hot-water oven kept at a constant temperature of about 27° C., this being a temperature which facilitates the parting of the oxygen from the permanganate of potassium.

In the presence of organic matter five-eighths of the oxygen is liberated from the permanganate in the following manner:

$$K_2Mn_2O_8 + 3H_2SO_4 + \text{oxidizable matter} = 2MnSO_4 + K_2SO_4 + 3H_2O + 5O \text{ (combined with oxidizable matter)}.$$

The amount of sulphuric acid added in the process is not sufficient to make the permanganate part with its oxygen, but merely to assist it in doing so in the presence of oxidizable organic matter.

4. After two hours remove the flasks from the oven, and proceed to estimate the amount of *undecomposed* permanganate. Add a drop or two of the solution of iodide of potassium, stirring well with a clean glass rod, when the pink colour is entirely replaced by a yellow one (due to free iodine). The undecomposed permanganate immediately reacts upon the iodide, with the result that an amount of free iodine is liberated proportionate to the amount of undecomposed permanganate, according to the following equation:

$$K_2Mn_2O_8 + 10KI + 8H_2SO_4 = 2MnSO_4 + 6K_2SO_4 + 8H_2O + 5I_2.$$

5. The next step is to ascertain the value of this free iodine in terms of sodium thiosulphate. Add by a graduated burette the standard solution of sodium thiosulphate until the yellow colour has almost completely disappeared—*i.e.*, very little free iodine remains; and so as to estimate the remaining trace with greater precision, create the blue colour of the iodide of starch by adding a drop or two of starch solution, then resume the addition of the standard solution of sodium thiosulphate until this blue colour has just disappeared. The reaction of the thiosulphate solution with the free iodine is according to the following equation:

$$2Na_2S_2O_3 + I_2 = 2NaI + Na_2S_4O_6.$$

If this process of decoloration has been properly performed, and if the necessary amount of thiosulphate solution has not been exceeded, a drop of the permanganate solution will suffice to restore the blue colour to the water.

6. When we come to similarly estimate the free iodine of the control test in terms of the thiosulphate solution, the quantity of the latter used will be the amount which is equivalent to 10 c.c. of the standard solution of permanganate (containing 1 milligramme of available oxygen). The difference, therefore, between the amount of thiosulphate solution here required and that required to titrate the amount of free iodine liberated by the permanganate in the sample of water will represent the amount of permanganate decomposed.

Example.—The *distilled* water + 10 c.c. of permanganate used up 26·5 c.c. of the thiosulphate solution.

∴ 26·5 c.c. of the thiosulphate solution may be considered as equivalent to 10 c.c. of permanganate, or the 1 *milligramme* of *oxygen* which this will part with.

The *sample* water + 10 c.c. of permanganate required only 24·5 c.c. of the thiosulphate solution, and therefore an amount of oxygen equivalent to 26·5 − 24·5 = 2·0 c.c. of thiosulphate solution has been taken up by the organic matter. But if 26·5 c.c. of thiosulphate solution is equivalent to 1 milligramme of oxygen, then 2·0 c.c. = 0·075 milligramme of oxygen.

∴ 0·075 milligramme of oxygen is taken up by 100 c.c. of water (100,000 milligrammes); or the oxidizable organic matter in a *hundred thousand parts of water* required 0·075 *part of oxygen to oxidize it* in two hours at 27° C.

Notes.—It is essential to bear in mind the important fact that there are other substances which water is liable to contain which will reduce the permanganate besides organic matter—*i.e.*, nitrites, ferrous salts, and sulphur compounds other than sulphates—so that it is necessary to dispose of or account for these before attributing the reduction in the permanganate solely to oxidizable organic pollution. Since as little as ¼ grain to the gallon of iron can be detected by the chalybeate taste which it imparts to the water, in the absence of any such taste the presence of iron may be disregarded. If, however, iron is markedly present as a ferrous salt, one may deduct from the total oxygen consumed the amount necessary to convert the iron into the ferric condition (112 parts of iron in a ferrous form will require to this end 16 parts of oxygen). To get rid of the nitrous acid and sulphur compounds other than sulphates, it is necessary to boil the water after acidulation with the sulphuric acid for about twenty minutes. Then it is made up to the original bulk with

distilled water, allowed to cool to 27° C., and the test is then proceeded with as above.

The amount of permanganate added must always be sufficient to leave a distinct pink colour at the end of the heating. Therefore, in some foul waters it is necessary to make further additions of the permanganate solution, carefully noting the *total* amount which has been employed. The calculation is facilitated if in these cases the same amount is added to the control flask.

At the end of the process—*i.e.*, after titration—the blue colour returns when the fluid has been exposed a few minutes to the air.

Conclusions to be Drawn from the Amount Estimated.—In *very pure* waters the oxygen thus absorbed in two hours is below 0·05 part per 100,000; but a figure not exceeding 0·1 is not unfavourable to the water's purity. Even when the latter figure is exceeded no definite conclusion can be come to unless the nature of the organic pollution is known, since a *peaty* water would not necessarily be judged as harmful even if it required three or four times this amount of oxygen to oxidize its vegetable organic matter.

The following table of approximate standards for this process was drawn up by Frankland and Tidy:

AMOUNTS OF OXYGEN ABSORBED BY 100,000 PARTS OF WATER.

	Derived from Upland Surfaces.	Derived from Sources other than Upland Surfaces.
Water of great organic purity	Not more than 0·1	Not more than 0·05
Water of medium purity	,, ,, 0·3	,, ,, 0·15
Water of doubtful purity	,, ,, 0·4	,, ,, 0·2
Polluted water	More than 0·4	More than 0·2

E. FRANKLAND'S PROCESS

A short reference only to this ingenious process is here given, since it is too difficult and complex for any but trained chemists to perform, and it is generally held that Wanklyn's method attains to as true an estimate of the organic matter at the cost of far less trouble; and as regards the opinion which the results enable one to form upon the water, it *closely* coincides with that formed when the same water is analyzed by Wanklyn's method. The process has been employed in certain official analyses, and

the public health student should understand the significance of the terms used to express results.

As pointed out on a previous page, the rationale of the method is as follows: When water is evaporated to dryness, and the residue is burnt with the oxide of copper, the nitrogen and the carbon which result from the combustion of the organic matter can be collected and estimated as "organic nitrogen" and "organic carbon." Steps are taken, of course, to eliminate the nitrogen and carbon which are not present in the form of organic matter.

The chief objections raised against the process are that it is tedious, costly, and difficult of performance. Inferences are drawn from the ratio which "organic nitrogen" bears to "organic carbon," while the amount of the former is less reliably estimated than that of the latter. Dupré points out that sea-water shows a ratio between the two worse even than is found in pure sewage.

By this process the purity of water is judged from a consideration of the *actual amounts* of organic carbon and organic nitrogen present and their *relative proportions* to each other; and both a low quantity of each and a small relative amount of organic nitrogen to carbon is favourable to the water. Much C and little N is indicative of vegetable pollution; whereas, if the relative proportion of N to C is high, the inference is that the pollution is largely of animal origin.

The Rivers Pollution Commissioners held that "a good drinking-water should not yield more than 0·2 part of organic carbon, or 0·02 of organic nitrogen in 100,000 parts." They found that in peaty waters the ratio of nitrogen to carbon was 1 : 11·9, while in similar waters that had been stored in lakes the nitrogen to carbon=1 : 5·9. In sewage the average of a large number of samples gave nitrogen to carbon=1 : 2·1. Highly polluted well-waters gave nitrogen to carbon=1 : 3·1.

CHAPTER XII

OXIDIZED NITROGEN (NITRATES AND NITRITES)

NITRATES and nitrites in water represent the oxidized nitrogen derived, in the main, from the decomposition of nitrogenous organic matter. When organic matter undergoes decomposition, much of the N passes off in the free state, the remainder combining with hydrogen to form ammonia; hence, when "free and saline ammonia" is found in large quantities in a water, it almost invariably affords evidence of the presence of very recent organic pollution, such as raw sewage. As the water continues on its course, the N, mainly through the action of so-called "nitrifying organisms" in the soil, becomes partially oxidized to nitrous acid (HNO_2), which, combining with bases (commonly of lime and less often of soda and potash), forms *nitrites*; therefore the presence of these salts generally indicates recent organic pollution. The same purifying agencies continuing to act, the nitrous acid combines with more oxygen, and becomes nitric acid (HNO_3), which forms nitrates of the above-mentioned bases, until ultimately none of the original N may have escaped this complete oxidation.

When both forms of ammonia by Wanklyn's process are very low, then practically the whole of the organic matter may be considered as thus purified; when this is not the case, however, purification has only been partially effected. When, as in some rare cases, the water in its subsequent flow meets with reducing agents (either inorganic or organic), the nitrates which have been built up may become gradually deoxidized, and reduced through nitrites to ammonia again; but in these cases one finds practically no albuminoid ammonia, oxidizable organic matter, etc., so that the large amount of free ammonia would not be taken as due to recent animal pollution.

It is necessary, however, in all cases where nitrates exist,

before ascribing their presence to *relatively* recent organic pollution (which will be almost entirely of animal origin), to preclude the possibility of their origin from soluble nitrates derived from remote organic matter in the strata permeated, since waters of great organic purity from the chalk, the oolite, the red sandstone, and the lias may contain marked traces. It has been suggested that these nitrates may sometimes be derived from fossil remains.

An appreciation of these facts enables a true estimate of the importance of the presence and amount of nitrates to be made.

Thus nitrites either indicate the incomplete nitrification of ammonia, or the reduction of nitrates by mineral reducing agents or microbes; thus, when they occur in shallow wells or rivers, their presence should suffice to condemn the water for drinking purposes, since they would point to the probability that animal pollution is present or very recent; but when they occur in deep-well water they *may* not denote present danger, for they may result from the reduction of nitrates by iron in natural deposits or even by iron pipes. Generally speaking, the importance to be attached to their presence will depend upon the results obtained from Wanklyn's process. Nitrites have, of course, a tendency to become nitrates, so that, whereas a water often contains the latter without any evidence of the former, nitrates will always be found accompanying nitrites; and, owing to their instability, it is exceptional to find nitrites in polluted samples of water.

Nitrates and nitrites exist only in traces in waters vitiated by vegetable matter alone, and plant life tends to remove nitrates and nitrites from a water; thus a polluted water, subsequently exposed to plant life, may furnish in its oxidized nitrogen but slight evidence of its previous pollution.

Even when the whole of the N of sewage matter is fully oxidized to nitrates, the water must be regarded as dangerous for drinking purposes, for at any time the agencies responsible for the purification may be overtaxed, and dangerous pollution may pass unchanged into the water.

Traces of nitrates are present in almost all waters, including rain-water.

Qualitative Tests for Nitrates.

The old **brucine test** in careful hands will detect extremely faint traces.

A few drops of a saturated solution of brucine are well mixed with half a test-tubeful of the suspected water; then, with the test-tube held well on the slant against a white background, *pure* sulphuric acid is poured gently down the side until the acid forms a distinct layer at the bottom of the test-tube. When the test-tube is brought to the vertical, a pink zone is seen to occupy the line of junction between the mixture of brucine and water and the sulphuric acid; the pink is transitory, however, and soon changes to a brownish-yellow. Especially does the colour change quickly when the nitrates are high in amount.

If no coloured zone appears, the test-tube should be gently swayed to and fro, so as, without mixing them, to bring more of the water and brucine in contact with the sulphuric acid; if the results are still negative, nothing but an insignificant trace of nitrates can be present.

A control test should be made with nitrate-free water, in order to test the purity of the sulphuric acid.

A still more delicate mode of applying the same test is to place 5 c.c. of the water in a perfectly clean platinum dish and evaporate to dryness. Then a drop of pure sulphuric acid is allowed to fall into the dish, and a minute crystal of brucine is added. A pink colour will appear with an extremely faint trace.

The Diphenylamine Test.—The sensitiveness of this test depends greatly on the mode of performing it. The reagent to be employed is a solution of diphenylamine in sulphuric acid and 5 per cent. hydrochloric acid; three or four drops of this reagent are added to 1 c.c. of the liquid to be tested, then 2 c.c. of concentrated sulphuric acid, and the whole shaken. In the presence of nitric acid or nitrous acid the mixture acquires a blue colour. When nitrites are present, the blue colour appears at once, whereas it forms slowly when due to nitrates. No other constituent of natural water gives a similar reaction.

Most tests for nitrates are responded to equally by nitrites. The brucine and sulphuric acid test responds also to nitrites, but not to nitrites in the absence of nitrates if the acid is diluted with an equal amount of distilled water.

QUALITATIVE TESTS FOR NITRITES.

The old **starch test** for nitrites is sufficiently reliable and delicate, when carefully performed, for most purposes; but there must be no sulphuretted hydrogen in the water. It consists in the addition of a little clear starch solution, and a drop of a solution of potassium iodide to some of the water in a test-tube. Dilute sulphuric acid is then added, when in the presence of nitrites a dark blue tint appears *immediately*, nitrous acid being liberated by the sulphuric acid; it then oxidizes the potassium iodide, leaving the iodine free to combine with the starch as the *blue* iodide. The test should be performed at the lowest possible temperature, and an instant reaction must take place, for nitrates give similar results after standing awhile.

Ilosvay's naphthylamine test is more delicate. The following solutions are required:

(*a*) Solution of sulphanilic acid, 0·5 gramme in 150 c.c. of dilute acetic acid (specific gravity, 1·04).

(*b*) Solution of naphthylamine, made by dissolving 0·1 gramme in 20 c.c. of distilled water, filtering, and adding 150 c.c. of dilute acetic acid.

If 1 c.c. of each of the above solutions be added to 50 c.c. of the suspected water in a Nessler glass, placed upon a white porcelain slab, a pink colour develops if nitrites are present. If no colour appears within fifteen minutes, nitrites may be considered as absent.

This method is sufficiently sensitive for ordinary purposes; but the most delicate appreciation of nitrites is made by acidifying a large bulk of the water with acetic acid, and then testing a little of the first part of the distillate from the water. If the water contains sulphuretted hydrogen, this must first be separated by means of a little well-washed carbonate of lead and subsequent filtration.

THE QUANTITATIVE ESTIMATION OF NITRITES.

The estimation may be based upon Ilosvay's reaction, the degree of colour thereby furnished being matched by means of a standard solution of potassium nitrite, in the manner of the colorimetric estimation of lead, as previously described.

The standard solution of potassium nitrite is made of the required strength by dissolving 1·1 grammes of pure silver nitrite in hot distilled water, and then adding a slight excess of potassium chloride. This solution is allowed to cool, and is then made up to 1 litre; the silver chloride is allowed to settle, and each 100 c.c. of the clear supernatant liquid is diluted to 1 litre; 1 c.c. of this liquid contains 0·01 milligramme of N as nitrite. The solution should be kept in the dark in a number of small bottles filled to the level of the stopper.

The quantity of nitrite present is estimated by taking several cylinders containing known amounts of standard nitrite solution, varying, say, from 0·02 to 0·1 milligramme of N as nitrite in 100 c.c. of distilled water; and 1 c.c. of each of the two solutions employed in Ilosvay's test must be added to the sample and comparison waters *at the same time,* since the colour gradually deepens upon standing. As the colour takes nearly a quarter of an hour to fully develop, the cylinders should be covered and set aside for this period before they are compared.

Example.—The comparison cylinder containing 8 c.c. of the standard nitrite solution is found to have the same tint of colour as that produced by the nitrite in the sample, and therefore the amount of N as nitrite in the sample of water is equivalent to that contained in 8 c.c. of the standard solution.

But 1 c.c. of this=0·01 milligramme of N as nitrite.

∴ 8 c.c.=0·08 milligramme of N as nitrite.

∴ there is 0·08 milligramme of N as nitrite in 100 c.c. (or 100,000 milligrammes) of water, or 0·08 part of N as nitrite in 100,000 parts of water.

The starch, iodide, and zinc reaction may also be taken advantage of as a means of making a quantitative estimation on colorimetric principles.

If the sample is coloured, it must be decolorized as much as possible by adding to 200 c.c. of the water 3 c.c. of a solution of sodic carbonate (1 : 3) and 1 c.c. of soda lye (1 : 4), when in most waters the precipitated carbonates of the alkaline earths carry down with them much of the colouring matter. If the water is soft, a few drops of a solution of alum should first be added.

As distilled water will often give a reaction for nitrite, care must be taken to see that the distilled water employed in the standard solution and in the comparison cylinders does not so react.

THE QUANTITATIVE ESTIMATION OF NITRATES AND NITRITES.

A reliable method is that known as the **copper-zinc couple process,** by which all the oxidized nitrogen in nitrates and nitrites is reduced to ammonia by a wet copper-zinc couple. The ammonia thus obtained can be distilled over and estimated in the manner described in Wanklyn's process. The process is not suited to the estimation of exceptionally large quantities of nitrates, but it is very accurate up to 1 part of N as nitrates and nitrites per 100,000, and is therefore applicable to the very large majority of waters which are examined as to their fitness for drinking purposes. Purvis and Courtauld have shown that the method is unreliable if organic substances yielding ammonia on reduction are present.

The Process.

1. A wet copper-zinc couple is prepared by taking a clean and bright piece of thin, well-crumpled zinc foil, and well cleansing this with dilute sulphuric acid. Then the zinc foil, which should measure about 9 square inches, is covered with a saturated solution of copper sulphate, and very quickly the surface of the zinc loses its bright appearance and becomes covered with a black adherent coating of metallic copper. As soon as this coat has thoroughly formed—and generally about three minutes will suffice—the zinc is removed, or the coating becomes pulverulent and falls away. It is then well washed with distilled ammonia-free water. The wet copper-zinc couple is placed in a thoroughly clean 8-ounce glass-stoppered bottle with a wide mouth, in order that it may take the "couple," and 110 c.c. of the water are poured in so as to cover the "couple," when the bottle is tightly stoppered and left all night in a dark, warm place (about 20° C.).

With very soft water a trace of sodium chloride should be added (about 0·1 gramme); and with very hard waters a small quantity of pure oxalic acid, to precipitate lime.

2. On the following morning 10 c.c. of the water should be removed and tested for nitrous acid by Ilosvay's test; the absence of this acid proves the completion of the reducing process, and its presence demands that the reaction should be given more time in which to complete itself.

3. In the absence of nitrites the remainder of the water (100 c.c.) is decanted into a boiling-flask, the bottle is well

washed out with ammonia-free distilled water, the washings being also added to the flask, and then about 400 c.c. of ammonia-free water are added.

4. The water is next distilled until all the ammonia present has come over. This is then Nesslerized as in Wanklyn's method, and the *nitrogen* present is calculated from the ammonia.

The molecular weight of ammonia being 17, and the atomic weight of N 14, N$=\frac{14}{17}$ of the ammonia estimated.

Of course, the amount of free ammonia originally present in 100 c.c. of the water (and which has already been estimated by Wanklyn's method) must be deducted from the total ammonia produced by this process.

Example.—The water furnishes 0·25 milligramme of ammonia, and since all of this must have been yielded by 100 c.c. of sample, 0·25 part per 100,000 is present.

But by Wanklyn's method the water showed 0·008 part per 100,000 of free and saline ammonia as originally present.

After deducting this amount, there is (0·25−0·008=) 0·242 part of ammonia due to nitrates and nitrites in 100,000 parts of the water sample.

The results are expressed in terms of "*nitrogen as nitrates,*" or as "*nitrogen as nitrates and nitrites*" in those cases where nitrites are also present. Nitrogen has been seen to form $\frac{14}{17}$ of ammonia; therefore there are $\frac{14}{17}$ of 0·242=0·199 of "*nitrogen as nitrates,*" or of "*nitrogen as nitrates and nitrites,*" as the case may be, in 100,000 parts of water.

If in those cases where nitrates co-exist with nitrites it should be desired to express the nitrogen of the *nitrates* alone, the nitrogen yielded by *nitrites* may be deducted. Assuming that the water has been found by the Ilosvay colorimetric method to contain nitrogen as nitrous acid to the extent of 0·029 part per 100,000, then 0·199−0·029=0·17 is the amount furnished by nitrates alone in 100,000 parts.

The ammonia thus furnished is generally in considerable quantities, and the colour in the first one or two Nessler glasses of distillate cannot on this account be directly matched by the chloride of ammonium solution; it can best be estimated by mixing the distillate collected in five Nessler glasses, adding 10 c.c. of Nessler reagent, and then matching the colour in an aliquot part, as previously recommended (see Wanklyn's Method), and then calculating.

The phenol-sulphonic acid colorimetric method of estimating nitrates is not quite so exact as the copper-zinc couple process, but the results can be very much more rapidly arrived at. The lesser delicacy of the process results only in a very slight error of under-estimation, which does not affect one's judgment upon the water.

The **Special Reagents required** are:

1. Phenol-sulphonic acid, made by mixing 6 grammes of pure phenol, 3 c.c. of distilled water, and 37 c.c. of pure sulphuric acid. Digest for several hours at 82° C. Preserve in a tightly stoppered bottle.
2. A standard solution of potassium nitrate (0·721 gramme to the litre), each c.c. of which contains 0·1 milligramme of nitrogen. Dilute tenfold, so that each c.c. = 0·01.

The Process.

1. Ten c.c. of the water sample and 10 c.c. of the standard nitrate solution are each placed in clean platinum dishes and almost evaporated to dryness.

2. Three c.c. of the phenol-sulphonic acid are then run into the dishes, which are subsequently placed on the water-bath for about five minutes.

3. The contents of the two dishes are poured into two separate Nessler glasses, and the dishes are carefully washed out with 25 per cent. ammonia solution. The washings of each dish are added to the Nessler glass which originally received its contents, and then more 25 per cent. ammonia solution is cautiously added to each glass until a yellow colour remains.

4. The contents of the glasses are then filtered (if necessary), and made up to 50 c.c. with distilled water. The Nessler glass containing the standard solution assumes a distinct yellow colour (due to the formation of potassium nitrophenol-sulphonate), and the contents of the other Nessler glass are also coloured, more or less, in proportion to the amount of nitrate in the 10 c.c. of water sample.

By transferring measured quantities from the deeper coloured liquid (which will almost always be that containing the potassium nitrate standard solution) into other Nessler glasses, which are again filled up with distilled water to their marks, a match is obtained; thus it is learnt how much of the deeper coloured liquid is required, when diluted to the 50 c.c. mark with distilled water, to match the tint in the cylinder with the less colour; or the darker solution may be poured into a measuring glass and

successive additions of water made until 50 c.c. poured into a Nessler glass is found to effect the match.

Suppose that 5 c.c. of the 50 c.c. of the darker coloured (standard) liquid effect a match. Then, since the colour in the whole of the standard liquid represents 10 c.c. of the standard solution of nitrate of potassium, the colour created by nitrates in the 5 c.c. $=(\frac{5}{50}$ or $\frac{1}{10}$ of the 10 c.c.) 1 c.c. of the standard solution.

But 1 c.c. of the standard solution contains 0·01 milligramme of nitrogen as nitrate; therefore there is 0·01 milligramme of such nitrogen in 10 c.c. (10,000 milligrammes) of water, or 0·1 milligramme in 100,000 milligrammes of water, or 0·1 part per 100,000.

If the sample is darker than the standard, then measured quantities of the sample must be removed and made up to 50 c.c. with distilled water until a match is obtained—*e.g.*, supposing 25 c.c. suffice for the match, then the sample cylinder contains twice as much oxidized N as the standard cylinder; and therefore the 10 c.c. of original water contained 0·2 milligramme of N as nitrates, or 2 parts per 100,000.

Nitrites slightly add to the colour formed in this process, and chlorides interfere with the delicacy of the estimation by furnishing lower results. If chlorides exceed 5 parts chlorine per 100,000 in the original water, a trace of pure sodium chloride should be added to the standard solution of nitrate of potassium.

Conclusions to be Drawn from the Amount Estimated.—The significance of the presence of nitrites and nitrates has already been discussed; and it has been seen that high nitrate indicates previous pollution, either distant and old or near and recent. When the "nitrogen in nitrates" exceeds 0·1 part per 100,000, suspicion is certainly justified in those cases where the strata may be excluded as the source from which the water may have derived such nitrogen; but where such a source cannot be excluded, an amount exceeding 0·5 would be regarded as suspicious, for it is exceptional that more is derived from entirely harmless sources. More than 0·1 part per 100,000 in rain or upland surface waters is therefore significant of animal contamination. No hard limits, however, can be accepted for all waters, and the amount of oxidized nitrogen must be considered in conjunction with the results of the other processes that help to furnish evidence of contamination.

CHAPTER XIII

THE GASES IN WATER

It is the aeration of water which furnishes its pleasant taste and sparkling appearance. The degree of aeration—as has been already pointed out—affords no evidence of the water's purity or impurity, since the foul water of a shallow polluted well is frequently markedly aerated, whereas the pure water collected from great depths is sometimes poorly so.

Rain-water, when thoroughly aerated, contains about 20·73 c.c. of gases per litre—*i.e.*, nitrogen 13·08, oxygen 6·37, and carbonic acid 1·28 c.c.

In addition to the innocuous gases upon which the aeration of a pure water depends—*i.e.*, nitrogen, oxygen, and carbonic acid—it is obvious that water may take up noxious gases, or those which, as they are generally the products of organic decomposition, may indicate danger (such as sulphuretted hydrogen, ammonia, marsh gas (CH_4), etc.).

To ascertain whether **free carbonic acid** exists in the presence of bicarbonates, a solution may be used of 1 part rosolic acid in 500 parts of 80 per cent. alcohol (to which baryta water has been added until it begins to acquire a red tint); when $\frac{1}{2}$ c.c. of this is added to 50 c.c. of water, no change takes place if free CO_2 is present, but a distinct reddening occurs in the absence of free CO_2.

The Lunge-Trillich method of estimating the free carbonic acid is very easy and accurate.

When Na_2CO_3 is added to water containing free CO_2, sodium bicarbonate is formed ($Na_2CO_3 + CO_2 + H_2O = 2NaHCO_3$), and when all the free CO_2 is combined the water reacts alkaline to phenolphthalein. The amount of Na_2CO_3 used may therefore be made to indicate the amount of CO_2 present.

One hundred c.c. of the sample are mixed with a few drops of a neutral alcoholic solution of phenolphthalein and titrated in a narrow glass cylinder with a $\frac{N}{20}$ solution of sodium carbonate,

until a faint, but permanent, red tint appears. This gives the amount of free CO_2.

Example.—2·2 c.c. of $\frac{N}{20}$ Na_2CO_3 were required to neutralize the free CO_2 in 100 c.c. of water.

One c.c. of $\frac{N}{20}$ Na_2CO_3 contains $\left(\frac{0·053}{20}=\right)$ 0·00265 gramme of Na_2CO_3.

But 106 parts of Na_2CO_3 neutralize 44 parts of CO_2.

∴ One c.c. of $\frac{N}{20}$ $Na_2CO_3=\frac{44}{106}$ of 0·00265=0·001 gramme of CO_2.

∴ 2·2 c.c. of $\frac{N}{20}$ Na_2CO_3=2·2 × 0·001 gramme CO_2=0·0022 gramme of CO_2.

∴ 0·0022 gramme CO_2 in 100 c.c. water, or 2·2 parts per 100,000.

CO_2 as Carbonate and Bicarbonate (Thorpe's Method):

1. Take 100 c.c. of water in a flask and add a drop or two of phenolphthalein.

2. Add standard oxalic acid solution (2·863 grammes of pure recrystallized oxalic acid to the litre of distilled water, 1 c.c. of which equals 1 milligramme of CO_2) until the phenolphthalein is decolorized, carefully noting the amount of solution used. This will indicate the CO_2 present as carbonate.

3. Next boil the water for about ten minutes, and then note the amount of standard acid required to decolorize.

When the acid is first added to the water the carbonates are converted into bicarbonates, and when this conversion is complete the phenolphthalein is decolorized.

When the water is boiled CO_2 is driven off, and the converted bicarbonates and the original bicarbonates are reduced to carbonates. The second titration will furnish the amount of CO_2 remaining after boiling. Twice this quantity will represent the total CO_2 as bicarbonates prior to boiling, and this amount less the original CO_2 in carbonates will furnish the amount of CO_2 as bicarbonates originally in the water.

Example.—One hundred c.c. of water required 2·5 c.c. of oxalic acid=2·5 milligrammes of CO_2 as carbonates, or 2·5 parts per 100,000.

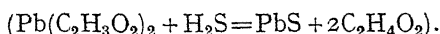
After boiling, 3·5 c.c. of oxalic acid were required. The total CO_2 as bicarbonates is therefore 7 milligrammes. Deduct the 2·5, and the amount in the original water was 4·5 parts per 100,000.

Sulphuretted hydrogen frequently gains access to water through organic substances undergoing putrefaction, or indirectly from industrial waste matters. It may be derived from mineral sulphides, or from reduction of mineral sulphates in soil, etc. (which reduction is frequently effected by organic matter and living organisms, such as *Beggiatoa alba*).

Good examples of waters charged with sulphuretted hydrogen from harmless sources are to be found notably at Harrogate and Aix-la-Chapelle. The waters from some clays have a distinct amount of sulphuretted hydrogen, derived from the tiny particles of iron pyrites which enter into the composition of the clay.

When sulphuretted hydrogen, ammonium sulphide, or the constituents of coal gas are present, the water will generally be condemned as either polluted or otherwise unsuitable for a domestic supply.

If H_2S is in considerable amount, the addition of a solution of acetate of lead produces a brownish coloration—

$$(Pb(C_2H_3O_2)_2 + H_2S = PbS + 2C_2H_4O_2).$$

The gas may be estimated while in the water in the following manner:

Take a large flask and add 10 c.c. of centinormal iodine solution; then run in a measured quantity of the water until the yellow colour of the free iodine disappears ($I_2 + H_2S = 2HI + S$); then add 5 c.c. of starch solution, and run in more of the iodine solution cautiously until a blue colour just begins to show itself. Of the iodine solution used, each c.c. will have decomposed 0·17 milligramme of H_2S, and therefore the total will represent 1·7 milligrammes of H_2S. The slight excess of iodine required to produce the blue colour is trivial, but it may be estimated and deducted by titrating back with sodium thiosulphate.

Centinormal iodine (1·26 grammes iodine per litre) is prepared as follows:

Iodine is never quite pure, and is very volatile. Dissolve therefore about 1·3 grammes in a solution of 2 grammes of potassium iodide in 50 c.c. of water, and dilute to a litre; then further dilute until 10 c.c. of the solution, coloured blue with a few drops of starch solution, are decolorized by exactly 10 c.c. of centinormal sodium thiosulphate (2·464 grammes to a litre). The solution should be kept in the dark.

Evidence of odorous gases may be obtained by heating the water to 60° C. in the manner already described in reference to

"odour." A few drops of a solution of the nitro-prusside of sodium will distinguish between sulphuretted hydrogen and ammonium sulphide, for that solution furnishes a violet-purple colour with ammonium sulphide, but no change results if sulphuretted hydrogen alone be present.

Some waters issuing as springs in the vicinity of volcanoes are charged with sulphurous acid.

Winkler's Method for the Estimation of Dissolved Oxygen in Water.

In collecting the sample of water, care must be taken to avoid agitating it and exposing it for any length of time to the air.

1. A portion of the sample is transferred with the above-mentioned precautions to a glass-stoppered bottle of known capacity. A suitable capacity is about 300 c.c., and the bottle must be completely filled.

2. One c.c. of strong manganous chloride solution (40 grammes of $MnCl_2H_2O$ to 100 c.c. of distilled water) is added, followed by 2 c.c. of a solution containing 33 per cent. caustic potash and 10 per cent. potassium iodide.

3. The bottle is stoppered without including any air-bubble, and the liquids are mixed by several times inverting the bottle. The manganous hydroxide precipitate which forms will be more or less discoloured by higher hydroxide, according to the proportion of oxygen which was dissolved in the water sample.

4. As the oxidation of the manganous hydroxide is not immediate, and the result is influenced by light, the bottle is put aside in a dark cupboard for fifteen minutes; 2 to 3 c.c. of pure strong hydrochloric acid are then added by means of a pipette inserted into the bottle, so that the acid will fall upon the precipitate, when the precipitate disappears and leaves the liquid coloured with dissolved iodine, which is proportionate in amount to the higher hydroxide formed, and therefore to the dissolved oxygen in the water.

5. Pour the contents of the bottle into a clean beaker, washing out the bottle with distilled water and also adding the washings to the beaker, and then titrate the iodine with decinormal sodium thiosulphate, of which 1 c.c. is equivalent to 0·0008 gramme of oxygen. Starch should be used for the end reaction, as recommended in Tidy's process.

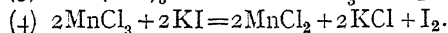
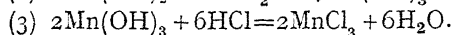
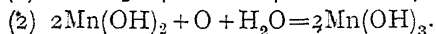
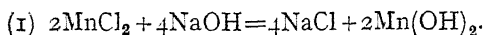
Example.—Capacity of bottle (283 c.c.) less the 3 c.c. of solution added=280 c.c. The decinormal sodium thiosulphate required in the test was 2·8 c.c.=0·00224 gramme of oxygen. Therefore there is 0·00224 gramme of dissolved oxygen in 280 c.c. of water=0·0008 gramme of oxygen in 100 c.c. of water= 0·008 gramme of oxygen in 1,000 c.c. of water, or 8·0 milligrammes per litre.

Notes on the Process.—Sometimes the amount of thiosulphate required by the same volume of fully aerated distilled water is determined, and the percentage of dissolved oxygen in the sample is compared with the amount of thiosulphate which equal volumes of these two waters require.

The manganous chloride must be free from iron, and all the reagents must be free from nitrites.

The process must be done rapidly. Nitrites liberate iodine, and so vitiate the result by increasing it. Much organic matter interferes with the method, for it absorbs liberated iodine, and thus diminishes the result.

The chemistry of the process is explained by the following equations:

(1) $2MnCl_2 + 4NaOH = 4NaCl + 2Mn(OH)_2$.
(2) $2Mn(OH)_2 + O + H_2O = 2Mn(OH)_3$.
(3) $2Mn(OH)_3 + 6HCl = 2MnCl_3 + 6H_2O$.
(4) $2MnCl_3 + 2KI = 2MnCl_2 + 2KCl + I_2$.

The amount of dissolved oxygen in a water is influenced by temperature, being less in summer and more in winter. Ordinary tap-water in this country contains between 6 and 7 c.c. per litre, or about 1 part by weight in 100,000. Water is saturated at 5° C., 10° C., 15° C., and 20° C. respectively, by 8·68 c.c., 7·77 c.c., 6·96 c.c., and 6·28 c.c. per litre.

When nitrites exceed faint traces the results are too high, owing to the reaction between the nitrous acid and hydriodic acid; the reaction is catalytic, the nitric oxide formed absorbing oxygen from the air and yielding nitrous acid, which in turn decomposes a further quantity of hydriodic acid. This effect may be prevented by carrying out the method in the usual way, and introducing 2 c.c. of potassium acetate solution (1,000 grammes per litre) when the precipitate has dissolved in the added hydrochloric acid. The acetate solution should be added by means of a pipette reaching to the bottom of the bottle (Hale and Melia).

CHAPTER XIV

COMPOSITION OF WATER FROM VARIOUS SOURCES—THE OPINION ON WATER SAMPLES

WATERS from the subsoil, from cultivated surfaces, and from rivers are especially liable to be organically polluted; and the character of the deposit from which the water is collected influences its composition to an extent which, though variable, may be approximately defined.

1. *Surface Waters.*—Those waters collected from the hard surfaces of the practically impervious rocks, which support little animal or vegetable life, are very pure. They commonly contain less than 10 parts of total solids, 5 of total hardness, 1 of chlorine, and 0·1 of nitrogen as nitrates, in 100,000 parts of water. The mineral solids consist mainly of sodium carbonate and chloride, and a trace only of lime or magnesia. The organic matter, which is often exclusively of vegetable origin (peat), yields practically no free ammonia; but the organic ammonia figure and that of the oxygen absorbed by organic matter may be high, in which case the water is often highly coloured and acid in reaction. Such characters are presented by the waters collected from the surfaces of the igneous, metamorphic (quartz, mica, granite, etc.), Cambrian, Silurian, and Devonian rocks.

Waters from the surface of the non-calcareous carboniferous rocks (Yoredale rocks, millstone grits, and coal-measures) are very similar; but those which have flowed over the surfaces of the calcareous carboniferous rocks—the mountain limestone and limestone shales—differ from the former in possessing a moderate degree of hardness, higher total solids, and a neutral or faintly alkaline reaction, the mineral solids consisting chiefly of sulphate and carbonate of calcium and magnesium.

Surface waters from the lias, new red sandstone, magnesian limestone, and oolite vary considerably in their composition

The total solids are generally between 10 and 20 parts per 100,000, the total hardness between 10 and 15; the chlorine is below 2, and the nitrogen as nitrates below 0·2 of a part per 100,000.

Clay surface waters are, as a rule, opaque from a variable quantity of suspended matter, but generally there are few dissolved solids, and the water is fairly soft. They vary greatly, however, in their composition.

Waters collected from cultivated land present great variations in composition; the total hardness may range from 5 to 25 parts per 100,000, according as to whether the soil is non-calcareous or calcareous.

Alluvium is generally a mixture of sand, clay, and organic matter; and waters from such a source mostly contain high mineral solids (50 to 100 parts), consisting of calcium and magnesium salts, sodium chloride, iron, and silica.

2. *Waters from a Depth.*—Those collected from the chalk are generally clear, bright, and well charged with carbonic acid. The total solids are generally from 25 to 50 parts per 100,000, and the total hardness from 15 to 30 parts; the hardness is mostly temporary, and calcium carbonate may vary from 10 to 30 parts. The chlorine is commonly from 2 to 4, but it may reach a higher figure in some pure chalk waters. The nitrogen as nitrates is generally below 0·5 part per 100,000, and is commonly about 0·2. Sulphates are present in small quantity, and there is often a trace of phosphates and of iron. Although the carbonic acid present may be sufficient to turn blue litmus red, when this gas is driven off by heat an alkaline action is invariably obtained.

Some waters from the chalk are very soft, and contain sodium carbonate. They are only found where the chalk lies buried beneath a thick mass of London clay (Thresh).

Waters from the oolite present characters very similar to those from the chalk.

Those derived from limestone and magnesium limestone formations only differ from the chalk waters in generally containing more total solids, far more calcium or magnesium sulphate (which may reach nearly 20 parts per 100,000), and less calcium or magnesium carbonate; and by consequence the hardness is generally higher and in a greater proportion "permanent."

In dolomite districts the mineral solids contain much magnesium carbonate and sulphate, and a large proportion of the

total hardness is " permanent," dolomite being a double carbonate of lime and magnesia.

The greensands are porous strata containing a reducing salt of iron, which, by reducing oxidized nitrogen to ammonia, often furnishes to the water a very high figure of free and saline ammonia. The total solids vary considerably, but they sometimes approach 100 parts per 100,000 where the water is collected at great depths in greensand underlying the chalk; the chlorine may reach a figure of from 4 to 15; the total hardness (much of which is " permanent ") is very variable—from a low to a high figure; and the nitrogen as nitrates is generally from about 0·3 to 0·6 part per 100,000.

Where the lower greensand is exposed it is very porous, and many of the waters yielded from it contain but little lime and hardness. The total solids are often small in amount, and the chlorine and saline constituents (including ammonia) may be low. A marked amount of nitrate is often present, and not infrequently a considerable quantity of iron is found in solution. Such waters differ materially from those obtained from the covered beds, the latter often containing large amounts of saline matter (chiefly alkaline chlorides, sulphates, and carbonates). This difference in the composition of the waters from exposed greensand and those from covered beds is probably accounted for by the explanation that the soluble constituents in the very porous exposed part of the bed have been largely washed out by the rapidly percolating waters.

Waters from red sandstone strata vary considerably in their composition, according as the deposit is pure or impure, soft or hard. The total solids and total hardness are both sometimes high, and the former may reach 100 parts per 100,000; the latter is mainly of a permanent nature, but the water may sometimes be soft and possess a total hardness figure not exceeding 10 parts per 100,000. The chlorine may vary from 2 to 6; and traces of phosphates are always to be detected in the mineral solids, which consist in the main of sodium chloride, carbonate and sulphate, calcium and magnesium carbonates and sulphates, and a trace of iron.

Waters from selenitic deposits are sometimes objectionable, on account of the large proportion of calcium sulphate (10 to 30, or more, parts per 100,000) which is taken up from this deposit, which itself consists of calcium sulphate in clear crystals.

Waters collected from loose sands are of variable composition. Some are soft, with total solids of from only 6 to 12 parts per 100,000, and others are rather hard (permanent), with mineral solids amounting to even 100 parts. The chlorine figure is generally rather high, and may reach to a very high figure in some cases. The mineral solids consist of sodium chloride, carbonate and sulphate, calcium and magnesium salts, and traces of iron and silica. Those from gravel are generally soft, but some are hard, with rather high total solids. Waters of the latter class coming from a depth have often very high mineral solids (often consisting largely of calcium and magnesium sulphate). There is, as a rule, some opacity, and the physical characters generally are not favourable to the water. The hardness, which is almost entirely "permanent," is often over 20, and the mineral solids may in some cases reach a high figure.

Deep wells, when protected from surface drainage and ground water in their upper parts, are but rarely polluted, even when situated in the centres of towns. But it does occasionally happen that liquid soakage from sewers or cesspools finds its way into fissures in chalk or sandstone, which conduct it to the water of the well, maybe from a considerable distance.

The Opinion on Water Samples.

If the analysis does not justify suspicion, and local circumstances do not favour any form of dangerous contamination, then, and then only, may the water be judged safe for drinking purposes.

Either a chemical analysis or a bacterioscopic examination may alone suffice to demonstrate the fitness of a water for drinking purposes, but the two processes are complementary to each other, and it is essential in many cases that they should both be performed. Neither is infallible; the value of one is often greatly enhanced by the other; and the value of both depends upon the correct interpretation of results.

Between an undoubtedly bad water and an undoubtedly good water there are waters regarding which no opinion ought to be advanced unless advantage is taken of a careful local inspection to detect any possible source of pollution, and both a bacteriological examination and a chemical analysis are performed.

It is true that so small an amount of organic matter as would not call for condemnation of the water may yet contain the

specific germs of disease; but chemical analysis will generally reveal impurity and risk. As specific germs always gain access to the water in the media of dirt and animal matter, it is rare that the chemical examination fails to indicate the danger; but whenever there is any reason to suppose that a water-supply is infected with typhoid or cholera organisms, a bacterioscopic examination becomes imperative. Indeed, the circumstances which call for the analysis of a particular sample should always weigh with one in fixing the standard of purity which will justify the opinion that the water may be consumed with safety.

The detection of a very fine amount of organic contamination in shallow wells may often be made by collecting samples from several wells in the near neighbourhood of each other, and taking the purest of these waters as a standard.

In judging of the purity of water from a river or small stream, it will sometimes be advantageous to make an analysis of tributary streams emptying into it.

Water from different sources (peaty and non-peaty surface waters, waters from shallow and deep sources) have their own characteristics, and the various quantitative estimations must be interpreted accordingly. It is impracticable to lay down hard-and-fast standards of purity (either chemical or bacteriological) to which all waters must conform, irrespective of their source.

In expressing an opinion upon the analytical results, it is very desirable to give expression to the necessary limitations of the examination. Thus, it will be better to say, "The chemical analysis of the sample shows no evidence of harmful organic pollution," than to employ such a statement as, "There *is* no harmful organic pollution in this water." In order to commit oneself to a statement which would imply that the water-supply is a *constantly* pure one, it will often be necessary to examine several samples at different seasons of the year and under different conditions as to rainfall.

Water Standards.—It would often be of assistance to analysts if they carefully constructed "water standards" in their districts. Such would be prepared from the *purest* water in each locality, and they would form a reliable means of detecting the smallest amount of impurity which gains access to any particular supply. For instance, a water sample may well contain 0·004 part per 100,000 of free and saline ammonia and 2 parts per 100,000 of chlorine without any suspicion of danger being warranted; but if the average of the pure water of the particular

locality is 0·002 part of free and saline ammonia and 1·5 parts of chlorine, then the excess found would furnish important evidence of animal pollution.

In some of the American States, Massachusetts notably, the normal distribution of chlorine has been mapped out. This has been done by estimating the amount of chlorine in the unpolluted waters of the district at a large number of points, and thus ascertaining the normal distribution of chlorine in every particular locality. When lines are drawn upon the district map which join together the localities where the chlorine figures correspond, these lines are known as "isochlors." The plans of the "isochlors" are valuable, as when in a given spot an amount of chlorine is found in excess of the figure of the particular "isochlor" of the locality, it furnishes material evidence of sewage pollution.

Chlorine standards are of the most value when they relate to surface water, in which the small chlorine figure remains very uniform indeed in the absence of animal pollution; but even then they often fail to indicate harmful pollution. The superior value of the "free and saline" ammonia figure is very apparent in the following results:

	A. Water.	B. Sewage.	C. Water + $\frac{1}{10}$ per Cent. of Sewage.
	Parts per 100,000.	Parts per 100,000.	Parts per 100,000.
Free and saline ammonia	0·0005	6·2100	0·0067
Albuminoid ammonia ..	0·0035	1·3200	0·0048
Oxygen absorbed in 2 hours at 27° C.	0·0300	4 1430	0 0341
Total solids	30·1	150·0	30·25
Chlorine	1·9	10·2	1·91
N as nitrates	0·19	0·00	0·18

Thus the water polluted with $\frac{1}{10}$ per cent. of the crude sewage taken in the experiment (or 1 gallon of sewage to 1,000 gallons of water) shows the free and saline ammonia increased about 13 times, the albuminoid ammonia increased by not much over $\frac{1}{3}$ of its original quantity, the total solids remain practically the same, and *the chlorine shows no appreciable increase*.

When the health officer has to make *periodical* examinations of the same water, it will not be necessary, *in order to detect contamination*, always to perform a complete analysis. No object will be gained, for instance, by the estimation of the total solid matter and the hardness; but one must never neglect

the free and saline ammonia figure, which is of paramount importance. It may be said with certainty that in such cases the slightest increase of ammonia should be regarded with grave suspicion.

In the light of a chemical analysis the fitness of the water for drinking purposes will be determined by the following considerations:

Is any evidence forthcoming of animal contamination present or past? Is there evidence of excessive vegetable pollution? Is the nature and amount of the saline constituents likely to prove harmful? Are poisonous metals present?

The suitability of the water for other uses, such as for washing and trade purposes, will depend upon its figure of hardness, the absence of a marked colour, and freedom from suspended or deposited matter.

The evidence of *present* or *recent* animal pollution is more especially indicated by high chlorine and oxidized nitrogen, in association with *marked* free and albuminoid ammonia; and that of *past* or *remote* animal pollution by high chlorine and oxidized nitrogen (not accounted for by the strata permeated) with *little* free and albuminoid ammonia.

If the pollution is solely of a vegetable origin it is indicated by high figures of albuminoid ammonia and of oxygen absorbed by Tidy's method, in association with very low figures of chlorine and oxidized nitrogen in the case of surface waters, and with practically no increase in these latter two figures if the water is collected from below the surface. Waters containing excessive vegetable pollution would generally be coloured, and the solid residue would char considerably upon ignition. As to excessive or harmful mineral matter in a drinking-water, a limit of 100 parts per 100,000 ought not to be exceeded; but it is generally the nature of the mineral matter rather than its amount which will affect the opinion. Sulphates should not furnish more than 10 parts of SO_3 per 100,000; iron is only permissible when in traces, and other poisonous metals should be absent.

A water containing over 30 parts of hardness may be regarded as unfit for trade, washing, and cooking purposes. It is rare with these waters that the total hardness cannot readily be reduced considerably by a water-softening process.

A few examples of waters from different sources, together with the opinion upon them which the chemical analysis (expressed as parts per 100,000) appears to warrant, may now be given.

	1. A very Pure Water.		2. A Foul and Dangerous Water
Physical characters	Excellent	..	Excellent
Reaction	Faintly alkaline	..	Markedly alkaline
Free and saline ammonia	0·001	..	0·022
Albuminoid ammonia	0·002	..	0·014
O absorbed from permanganate (in two hours at 27° C.)	0·012	..	0·114
Total solid matters	18·0	..	38·4
(*a*) Volatile	4·6	..	18·1
(*b*) Fixed	13·4	..	20·3
(*c*) Appearance on ignition	Nil	..	Marked charring
Total hardness	9·0	..	24·0
(*a*) Temporary	4·0	..	16·0
(*b*) Permanent	5·0	..	8·0
Chlorine	1·0	..	6·2
N as nitrates	0·01	..	0·8

Note.—Sample 2 is evidently a chalk water contaminated with animal matter, as evidenced by the high ammonias (the "free" being higher than the "albuminoid"), and the high figures of oxidized nitrogen and chlorine (for a chalk water). The very high figure of free and saline ammonia points to recent and therefore specially dangerous contamination.

	3. Rain Water (Country).		4. Subsoil Water (Gravel over Chalk).
Physical characters	Good	..	Good
Reaction	Faintly alkaline	..	Alkaline
Free and saline ammonia	0·050	..	0·002
Albuminoid ammonia	0·005	..	0·006
O absorbed from permanganate (in two hours at 27° C.)	0·005	..	0·061
Total solid matters	3·0	..	29·2
(*a*) Volatile	1·5	..	12·2
(*b*) Fixed	1·5	..	17·0
c) Appearance on ignition	Nil	..	Slight charring
Total hardness	0·5	..	12·0
(*a*) Temporary	0·0	..	5·5
(*b*) Permanent	0·5	..	6·5
Chlorine	0·25	..	2·1
N as nitrates	0·02	..	0·2

Notes.—Sample 3. In industrial towns the reaction may be slightly acid, from the sulphuric acid in the atmosphere; and the water is a little different in other respects owing to further impurities taken up, such as soot, sulphur compounds, and increased ammonia. Thus the rain falling in Manchester has been found to contain 0·7 part per 100,000 of free ammonia, 0·03 of albuminoid ammonia, 4·7 parts of sulphuric acid, and 0·58 of hydrochloric

acid. Rain collected on the sea-coast has been found to contain as much as 5·4 parts per 100,000 of chlorine (chlorides).

Rain water which is collected in country districts after long periods of continuous rainfall provides the purest possible *natural* water. Its composition varies throughout the year somewhat.

Sample 4. The analysis does not furnish evidence of harmful contamination; but the ammonias suggest the presence of a little vegetable matter.

	5. A Peaty Surface Water.	6. A Non-peaty Surface Water on Millstone Grit.
Physical characters	Brownish-yellow	Nearly colourless
Reaction	Acid	Neutral
Free and saline ammonia	0·001	0·002
Albuminoid ammonia	0·022	0·004
O absorbed from permanganate (in two hours at 27° C.)	0·160	0·040
Total solid matters	12·0	5·5
(a) Volatile	9·5	2·0
(b) Fixed	2·5	3·5
(c) Appearance on ignition	Marked charring	Faint discoloration
Total hardness	3·0	2·5
(a) Temporary	0·0	0·0
(b) Permanent	3·0	2·5
Chlorine	0·7	0·8
N as nitrates	0·01	0·05

Note.—In many "peaty waters" the "organic ammonia" and the "oxygen absorbed" will be found to much exceed the amounts given above. Neither of the above analyses furnishes evidence of harmful contamination.

	7. Deep-well Water (from Chalk).	8. Deep well Water (from New Red Sandstone).
Physical characters	Excellent	Good
Reaction	Alkaline	Alkaline
Free and saline ammonia	0·006	0·001
Albuminoid ammonia	0·011	0·002
O absorbed from permanganate (in two hours at 27° C.)	0·084	0·012
Total solid matters	40·0	30·2
(a) Volatile	15·5	8·6
(b) Fixed	24·5	21·6
(c) Appearance on ignition	Marked charring	Nil
Total hardness	26·0	19·5
(a) Temporary	15·5	8·0
(b) Permanent	10·5	11·5
Chlorine	4·5	2·2
N as nitrates	0·6	0·3

Notes.—A deep-well water from the chalk may contain total solids up to 200 parts per 100,000, but such an amount is rare.

Sample 7 is a water the analysis of which warrants suspicion of organic contamination. Slight animal contamination is probable in such a water, having regard to the figures of the saline and albuminoid ammonia, the chlorine, and the oxidized nitrogen. A careful local inspection might detect the source of some pollution.

There is no reason to question the purity of Sample 8.

	9.	10.
	River Water.	New River Water (as supplied in London).
Physical characters	Good	Excellent
Reaction	Faintly alkaline	Faintly alkaline
Free and saline ammonia	0·009	0·001
Albuminoid ammonia	0·017	0·002
O absorbed from permanganate (in two hours at 27° C.)	0·099	0·014
Total solid matters	32·5	31·5
(*a*) Volatile	14·0	9·0
(*b*) Fixed	18·5	22·5
(*c*) Appearance on ignition	Marked charring	Nil
Total hardness	20·5	22·0
(*a*) Temporary	9·0	8·5
(*b*) Permanent	11·5	13·5
Chlorine	2·4	1·8
N as nitrates	0·4	0·2

Notes.—Sample 9. The composition of river water will always, of course, vary with the following circumstances:

1. The nature of the country through which the river courses, and which it therefore drains—*i.e.*, whether this be cultivated and manured or wild, whether there be much or little vegetation, and whether it be thickly or sparsely populated.

2. The amount of pollution by sewage, waste products of manufactories, etc., which gains access to the water.

3. The nature of the bed of the river, and of the strata through which any springs (which feed the river) rise.

4. The rapidity and smoothness of flow—*i.e.*, the more rapid and interrupted this is, the greater the powers of the river in the direction of self-purification.

No. 9 furnishes evidence of contamination.

No. 10 is a water of great purity.

	11. Deep Spring Water (from Greensand below Chalk).		12. Spring Water (from Chalk).
Physical characters	Excellent	..	Excellent
Reaction	Alkaline	..	Alkaline
Free and saline ammonia	0·030	..	0·001
Albuminoid ammonia	0·001	..	0·003
O absorbed from permanganate (in two hours at 27° C.)	0·020	..	0·019
Total solid matters	111·2	..	32·5
(a) Volatile	21·0	..	8·5
(b) Fixed	90·2	..	24·0
(c) Appearance on ignition	Nil	..	Nil
Total hardness	18·5	..	23·0
(a) Temporary	7·0	..	18·0
(b) Permanent	11·5	..	5·0
Chlorine	12·2	..	3·0
N as nitrates	0·4	..	0·2

Notes.—In Sample 11 the high amounts of saline ammonia, chlorine, and mineral matter so frequently present in pure waters from the lower greensand are shown. Sometimes the nitrates are higher than in this sample. The absence of recent animal pollution in this case is shown by the very low figure of albuminoid ammonia.

Sample 12 is a very pure water. The ammonias are quite low.

	13. Well Water from Carboniferous Limestone.		14. Well Water.
Physical characters	Excellent	..	Excellent
Reaction	Alkaline	..	Alkaline
Free and saline ammonia	0·005	..	0·001
Albuminoid ammonia	0·006	..	0·001
O absorbed from permanganate (in two hours at 27° C.)	0·066	..	0·008
Total solid matters	31·9	..	48·5
(a) Volatile	9·8	..	17·3
(b) Fixed	22·1	..	31·2
(c) Appearance on ignition	Slight charring	..	Nil
Total hardness	24·2	..	31·5
(a) Temporary	17·9	..	9·5
(b) Permanent	6·3	..	22·0
Chlorine	1·9	..	6·2
N as nitrates	0·3	..	1·8

Notes.—Sample 13. The ammonia figures indicate slight animal contamination.

Sample 14 furnishes evidence of *previous* (remote) sewage contamination in the high figures of N as nitrates and chlorine. The extremely low albuminoid ammonia figure shows that the organic matter has been almost completely mineralized. Nitrites were absent, but phosphates were markedly present.

	15. Chalk Water.	16. Peaty Water.
Physical characters	Excellent	Light brown tint; clear
Reaction	Alkaline	Acid
Free and saline ammonia	0·018	0·005
Albuminoid ammonia	0·010	0·022
O absorbed from permanganate (in two hours at 27° C.)	0·082	0·122
Total solid matters	62·7	15·6
(a) Volatile	23·2	12·0
(b) Fixed	39·5	3·6
(c) Appearance on ignition	Marked charring	Marked charring
Total hardness	33·5	3·0
(a) Temporary	22·5	0·0
(b) Permanent	11·0	3·0
Chlorine	6·8	1·5
N as nitrates	0·9	0·2

Notes.—Sample 15 is a chalk water polluted with animal matter, as evidenced by the high saline ammonia (along with a considerable amount of organic ammonia) and the high figures of chlorine and oxidized nitrogen. The hardness is also excessive, but this may readily be reduced to 11 parts by a water-softening process.

Sample 16 is a peaty water polluted with animal matter, as evidenced by the fact that the figures of the saline ammonia, the chlorine, and of the oxidized nitrogen, are excessive *for a peaty water*. It is a water possessing a considerable plumbo-solvent action.

	17.	18.
Physical characters	Brackish taste; blue-green tint	Excellent
Reaction	Alkaline	Alkaline
Free and saline ammonia	0·001	0·004
Albuminoid ammonia	0·006	0·005
O absorbed from permanganate (in two hours at 27° C.)	0·050	0·042
Total solid matters	249·2	34·0
(a) Volatile	32·8	11·0
(b) Fixed	216·4	23·0
(c) Appearance on ignition	Slight discoloration	Faint discoloration
Total hardness	Very high	23·5
(a) Temporary	—	13·0
(b) Permanent	—	10·5
Chlorine	109·5	1·9
N as nitrates	0·9	0·3

Notes.—Sample 17 is a deep-well water in the chalk contaminated by sea water. This is evidenced by the fact that the chlorine is enormously high and magnesium chloride is abundant. The well was near the coast,

and, prior to the contamination, the chlorine was 4 parts per 100,000 and the total hardness 24. The water is quite unfit for domestic uses on account of its excessive hardness, the brackish taste, the deposit it will give rise to in boilers and kettles, and the fact that it will impair the palatability of tea, coffee, etc.; and it is altogether unsuitable for washing and cooking purposes.

Sample 18 is a polluted water. The above figures would barely warrant such an opinion, but a previous analysis of the water from the same source gave the saline and organic ammonias as 0·002 and 0·003 respectively, and the chlorine and oxidized nitrogen as 1·8 and 0·20 respectively. Some pollution has therefore recently gained access to the water, and the sample is included to illustrate the value of periodical analyses of a water-supply in detecting intermittent pollution.

An examination of the bed of a waterway or pond may serve to furnish corroborative evidence of the sewage contamination of the water. Of such contamination there is little or no reliable indication by chemical analyses, nor does a low-power microscopic examination and a bacteriological examination supply valuable evidence; and unless the pollution is gross, it is not possible to conclude that a mud is contaminated with human excrement, either from chemical or bacteriological data.

If any parts of the bed are covered with gravel or large stones which are not clean, and especially if the greyish flocculent growths characteristic of certain sewage fungi are found to be attached to them or to any other part of the bed, and if the mud is of a dark colour and emits gas-bubbles and offensive odour on being disturbed, then there are good reasons for suspecting gross sewage contamination. It is common in these circumstances to find some opalescent floating bubbles, which have but little tendency to burst, on parts of the surface of the overlying water.

Although the organic matter in pond and river mud will be found on an ordinary microscopical examination to be largely in the form of unrecognizable débris, with only a relatively small (but variable) quantity of the vegetable structure of plant life distinguishable, a textile fibre or animal hair, etc., would indicate dangerous contamination (*vide* p. 153).

Water markedly contaminated with sewage or sewage effluent is unfit to be used by cows for drinking purposes; for, apart from the risk to the health of the animal, there is a danger of specific organisms of intestinal origin getting upon the teats and udders of the cows, and thereby into the milk in the process of milking.

Gerber and Sheldon both agree that dirty drinking-water

may give rise to impure and tainted milk. We know that improper food, such as fermented potatoes or cabbages, affects the taste and keeping qualities of cow's milk; and there is no reason why what applies to food should not apply to drink. It is, moreover, only reasonable to suppose that the drinking of polluted water is injurious to the cow as well as to the milk; and that the purer the food and water given to cows, the better both for the animal and for the milk she furnishes.

The presence of a small amount of domestic sewage in a stream of fair volume and flow is apparently not injurious to fish. Oysters, mussels, and cockles are tolerant of considerable sewage pollution, although there is evidence that oysters become scarcer and smaller in the presence of gross pollution; but in the case of these shellfish, their capacity to retain specific disease-producing bacteria when bathed in polluted water makes the consumption of them, when collected from such waters, a grave danger, the reality of which has been abundantly demonstrated in this and other countries.

Fresh-water fish generally are more affected by pollution from chemical wastes than by sewage; but they vary considerably in their susceptibility to sewage contamination. In experimenting upon the effect of the sewage contamination of a stream upon fish life, allowance must be made for this fact. Trout appear to be very susceptible, and they require to be kept in running water. Gold-fish, gudgeon, and roach (of which the latter two are very sensitive to various forms of pollution in water, while the former is relatively resistant) are suitable fish to experiment with. These may be kept in the contaminated water, while at the same time control fishes are kept in pure water; and by observation of their active movements, their food consumption, the healthy appearance of their eyes, fins, tails, etc., the weight of the survivors at the end of the experiment, and the rate of mortality, it is not difficult to learn from the comparative data collected whether the polluted water has proved inimical or not.

Bacteriological Evidence.

Like the chemical, the value of this evidence has certain limitations. As ordinarily performed, even the bacterial counts furnish evidence only of the discrete masses of organisms. There is no necessary relationship between these and the numbers of

separate organisms originally present, because with efflux of time the organisms, like other suspended particles, tend to agglutinate. Again, at the present day it is often impossible to recover or recognize the specific germ, even shortly after this has been experimentally added to water; and the organisms which denote sewage contamination are the same whether they are derived from the lower animals or from human beings. Yet, despite these facts, the results of a bacteriological examination of water samples, interpreted in the light of topographical circumstances, is generally of great value.

The Collection and Transmission of Samples.—Great care is required in the collection of the samples; even apparently trivial errors or omissions may entirely vitiate the result. Very precise and seemingly trifling directions must be given, unless the sample is collected by an expert.

For the ordinary examination 2-ounce (57 c.c.) glass-stoppered bottles are sufficient. When larger amounts are required, a Winchester quart bottle may be used. The bottles should be sterilized, with their stoppers loosely inserted, at 160° C. for one hour, and allowed to cool slowly.

If the specimen cannot be examined at once, and delay is unavoidable, the sample should be packed in ice, and then transmitted to the laboratory. Special apparatus have been designed for this purpose.

That figured on p. 121 is a convenient form. Two-ounce glass-stoppered bottles are used. Each of these, after thorough washing, and drying in the hot-air apparatus, has its stopper inserted, and is then placed in a tin into which it just slips. The bottom of the tin has a layer of cotton-wool and then a piece of asbestos cardboard.

Several thicknesses of asbestos cardboard are also fitted in the cover of the tin, so that when in place the bottle is firmly in contact with the asbestos above and below. The tins with their contained bottles are then sterilized in the hot-air apparatus. Labels are placed on the outside of the tins, and they are ready for use. The ice-boxes are made to just receive one, two, or four such tins. The tins are not opened after sterilization until immediately before the sample is taken.

To take samples from various depths, a number of different forms of apparatus have been devised. The ordinary collecting-bottle may, however, be also used for this purpose. It is tied

into a leaden cage, and lowered to the required depth by catgut or string attached to the cage. The loosened stopper is then removed by a jerk upon a second string previously tied to the stopper, and the sample collected.

In collecting samples from a reservoir, lake, or river, plunge below the surface before removing the stopper, thus avoiding scum and surface contaminations. If from a tap, allow the water to first run to waste for five to ten minutes. If from wells with a pump, pump away a considerable quantity of water

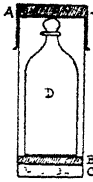



FIG. 15.—COLLECTING-BOTTLE AND TIN.

A, Asbestos cardboard in lid; B, asbestos cardboard below bottle D; C, cotton-wool layer.

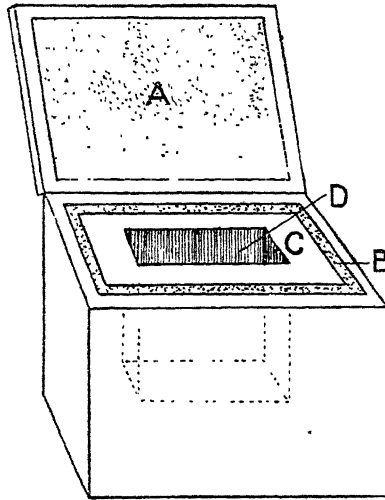



FIG. 16.—ICE-BOX.

A and B, felt lining; C, metal ice receptacle, with depression D, to hold two collecting-tins (with contained bottles).

before collecting the sample; while if a complete investigation is required, a second sample should be obtained after several hours' pumping.

Owing to the extreme difficulty of detecting the actual specific organisms of disease, such as the organisms of typhoid fever and cholera, it is necessary to resort to other methods of investigation which indicate the risk of specific infection.

Hygienists are unanimous in recognizing that sewage and the excreta of human beings, even when healthy, must be looked upon as potential vehicles for disease production. The presence

of the excreta of animals must also be looked upon as prejudicial, since it *may* contain harmful bacteria and other parasites.

A number of organisms have been advocated as fulfilling the requirements necessary for indicators of sewage contamination. Of these, *B. coli* and allied organisms, *B. enteritidis sporogenes*, and certain streptococci, are the only ones which have been extensively advocated and merit detailed consideration.

For these organisms it is not only necessary to ascertain their presence or absence, but, in addition, their numbers.

Significance and Interpretation of Results.—The detection of the cholera spirillum or the typhoid bacillus in a water, in whatever amount, is sufficient to condemn the water. The other results obtained in the bacteriological examination of water-supplies are, however, only data from which an opinion upon the purity or contamination of the water can be deduced with more or less confidence according to the data available.

Such deductions require much special experience, and for a detailed consideration of the matter the reader is referred to Dr. Savage's book on the subject,* limits of space allowing here only a brief summary and review.

The number of organisms developing upon gelatine plates is largely an index of the amount of organic matter in the water, although there is no constant or exact relationship between the two. Still, the addition of organic matter almost invariably means an addition both of foreign bacteria and of material which enables the water, for a time at least, to become a better nutrient medium, and so causes an increased proliferation of bacteria.

A low gelatine count is, therefore, a satisfactory feature; but, on the other hand, a high gelatine count cannot in itself be considered a sufficient reason for condemning a water. For surface waters the contamination is frequently with harmless organic matter, and of comparative unimportance.

Good deep-well and spring waters frequently contain less than 50 bacteria per c.c. developing on gelatine plates, while in surface waters, even when free from pollution, up to 500 or more per c.c. are not infrequently met with.

The blood-heat count (agar plates at 37° C.) is an index of the addition of bacteria other than those natural to pure water, but

* "The Bacteriological Examination of Water-Supplies" (H. K. Lewis, London, 1906).

they need not be harmful. The addition of harmless soil bacteria will cause a great increase in the number of the 37° C. organisms.

The number present in deep-water sources, when pure, is very low, frequently less than 1 per c.c., and 10 or more per c.c. is not satisfactory. In the case of surface waters and rivers, soil washings are common, and a more generous margin (50 to 100 per c.c.) is necessary. On the whole, a marked increase in the number of bacteria growing at 37° C. is of greater significance than a proportionate increase of the gelatine count.

Of much greater importance is the interpretation of the *B. coli* estimation. The views of different workers show considerable variance. This bacillus is abundant in human and animal excreta and in sewage, and it serves as a *measure* of excretal pollution.

Deep-well and spring water should not be liable to any pollution by material containing *B. coli*. Water from these sources, even if originally polluted, should have passed through a considerable depth of soil, and thus have become practically free from bacterial evidence of contamination. If such sources are properly protected at their outlets also, there is no reason why they should contain any *B. coli*. It is, therefore, justifiable to maintain an attitude of great suspicion towards any water from such sources which contains *B. coli* in 100 c.c. or less.

In the case of surface supplies and shallow wells the position is different. For example, considering upland surface waters, the opportunity for contamination by *B. coli* contained in animal (*e.g.*, sheep) excreta may be considerable. The *B. coli* from sheep excreta are indistinguishable from those from sewage or human fæces, yet no one would contend that they are of equal significance, or that it is equally important to prevent such contamination.

As a matter of experience, on the other hand, it will generally be found that *B. coli* (rigidly defined) is not found in shallow wells, or in the majority of surface supplies, in 10 c.c. or less, unless that water is being polluted with excrementitious matters in undesirable amount.

While, therefore, admitting that dogmatic standards are especially untrustworthy for these classes of waters, a working standpoint that the finding of *excretal B. coli* in 10 c.c. or less points to undesirable pollution is both justifiable and in accordance with actual experience. If no *B. coli* are present in 50 c.c. the

water may be safely passed as satisfactory. For rivers used as sources of drinking-water, without artificial purification, similar standards are applicable.

Sometimes the organisms isolated are not typical *B. coli*, but differing in the absence of one or more of the characteristic properties of this organism. In the opinion of most bacteriologists of experience, the nearer these lactose-fermenting coli-like bacilli approach typical *B. coli* in their characters, the more nearly are our numerical standards for that organism applicable to them, while if they lack essential characters a proportionately greater number must be present to justify an adverse opinion.

Determinations of the number of streptococci have been made much less frequently than in the case of *B. coli*. As a provisional guide, and without attaching an equal significance to the findings, a standard similar to that for *B. coli* may be employed—*i.e.*, their presence in 100 c.c. or less of deep-well or spring water, or in 10 c.c. or less of surface and shallow-well waters, would justify an adverse opinion as to the purity of the water in question.

On its negative side the streptococcus test is not of great value, and the absence of streptococci, even in a considerable bulk of water, cannot be taken as showing purity or freedom from danger.

Opinion is not united as to the value of *B. enteritidis sporogenes* as an indicator of pollution. It is fairly abundant in sewage and excreta, but it is a spore-bearing organism with prolonged powers of resistance, and therefore, even if it be admitted that its presence indicates pollution, such pollution may have taken place at some long antecedent period, a contamination so old as to be of no significance. But its absence in a large quantity of water is some evidence of purity.

It will be of assistance in the difficult matter of giving an opinion upon samples submitted for bacteriological examination if a few examples are given of samples from different sources. They represent actual analyses, in which the topographical conditions were accurately investigated either at the time of examination or subsequently.

1. An upland surface water collected in an open artificial reservoir:

Number of organisms developing per c.c. at 37° C. = 16.
" " " " " 21° C. = 224.
"Excretal" *B. coli* present in 40 c.c., but not in 10 c.c. or smaller amounts.
Streptococci absent in 50 c.c.

(Standard+1 media; incubation forty-two hours at 37° C., three days at 21° C.)

The bacteriological opinion from this sample would be favourable. Careful topographical investigation showed no evidence of any human sources of infection on the gathering ground, but the water was liable to some pollution from sheep's droppings, etc.

2. A mountain stream feeding a large upland surface reservoir:

Number of organisms developing per c.c. at 37° C.= 340.
,, ,, ,, ,, ,, 21° C.=1,640.
"Excretal" *B. coli* isolated from 10 c.c., but not found in smaller quantities of the sample.
Atypical *B. coli* isolated from 1 c.c.
Streptococci present in 30, 10, and 1 c.c., but not in 0·1 c.c.

The bacteriological evidence is here sufficient to condemn the water, and topographical investigation showed considerable opportunities for pollution from both inhabited houses and manured lands.

3. A surface (shallow well) provided with a pump:

Number of organisms developing per c c. at 37° C.= 112.
,, ,, ,, ,, ,, 21° C =7,700
"Excretal" *B. coli* isolated from 1 c.c., but not from 0·1 c c.
Atypical *B. coli* isolated from 0·1 c.c.
Streptococci present in 40 c.c., but not in 10 or 1 c.c.

The well is evidently polluted, and was, in fact, surrounded by manured ground, while the covering to prevent surface water gaining access was defective, and there was no internal rendering of the sides of the well.

4. A surface well provided with a pump:

Number of organisms developing per c.c. at 37° C.= 9.
,, ,, ,, ,, ,, 21° C.=710.
B. coli absent in 50 c.c.
Streptococci absent in 50 c.c.

Here the examination showed no evidence of any harmful contamination. The well was situated in a town, and was surrounded by houses, but surface water was prevented from entering. From the topographical survey it was impossible to say whether the water was polluted or not.

5. A spring used as a public supply:

Number of organisms developing per c.c. at 37° C.= 1.
,, ,, ,, ,, ,, 21° C.=34.
B. coli absent in 50 c.c.
Streptococci absent in 50 c.c.

The bacteriological results are quite satisfactory, and, indeed, showed very little variation at each monthly examination.

Careful investigation of the source showed no likely sources of contamination, but remote sources of pollution were possible, and systematic bacteriological examinations were necessary.

N.B.—The foregoing information under "Bacteriological Evidence" is, in the main, summarized from a contribution by Dr. W. G. Savage to previous editions of this book.

CHAPTER XV

SEA WATER

THE Rivers Pollution Commissioners found that sea water contains approximately:

	Parts per 100,000.
Total solids	3898·7
Chlorine	1975·6

A specimen collected by the late Dr. Tidy during high water at Margate gave:

	Parts per 100,000.
Total solids	3343·0
Chlorine	1770·5
Lime	35·1
Magnesia	205·6
Silica	0·4
Hardness	564·0

As edible sea shellfish, reared or deposited round our shores, are sometimes exposed to dangerously contaminated sea water, and sea water contaminated with human excrement has on good grounds been held to be responsible for the infection of enteric fever, it is desirable to learn what evidence is available of the sewage pollution of sea water.

A summary of our present position with reference to bacteriological evidence of contamination will serve to indicate the value of the assistance of chemical standards. Although the Royal Commission on Sewage Disposal, appointed in 1898, reported that they were satisfied that bacteriology could not at present be relied upon to determine whether or not shellfish are polluted by sewage, typical *Bacilli coli communis* in shellfish are usually regarded as sufficient evidence of such pollution; and in sea water, Houston, Hewlett, Klein, and others would take *B. coli communis* in 1 c.c. as indicating contamination. Dr. Houston's

work for the Commissioners, which was published in their Fourth Report, vol. iii., 1904, clearly shows that no sample of sea water remote from pollution contains either *B. coli communis* or the spores of *B. enteritidis sporogenes*, even when as much as 100 c.c. of the samples are used for test purposes. But birds and fish may contribute *B. coli communis*; and he suggests the prudence of not pushing an extremely delicate test too far. He further demonstrates that *B. coli*, added to sea water, is no longer in evidence in 1 c.c. of the sample after a maximum period of nine days and a minimum period of five days. He therefore concludes that absolute standards cannot be laid down at present; but he maintains that *B. coli* present in 10 c.c. and absent in 1 c.c. should be viewed with some degree of suspicion, the water not necessarily to be condemned apart from topographical and epidemiological considerations.

Such vegetable growths as *Ulva latissima* are not *delicate* indicators of sewage pollution, for they may not be in evidence in cases where sea water is exposed to the lesser degrees of contamination.

Of course, *gross* contamination is unmistakable when sea water is judged from the results of either a bacteriological or chemical examination; but it is the evidence of previous and relatively slight contamination that may be ill-defined and elusive. Coast-tides, currents, and eddies are capable of conveying sewage contamination for some distance from the actual outfall of sewage into the sea to parts where there is no local contamination added, and there is nothing to indicate such pollution. A period of twenty-four hours would suffice for this contamination, in a very dilute form, to reach several miles from its outfall; and laboratory experimentation indicates that the *B. typhosus* can survive several days in sea water.

The writer and F. N. Kay Menzies find that the chemical evidence upon which opinions are based as to the purity or otherwise of the various classes of fresh waters is not wholly applicable to sea water after slight contamination with sewage, and they conclude as follows:

While the chlorine figure is often a useful one for indicating animal contamination in *fresh* waters, it is useless in respect of *sea water*, for the reason that a relatively small amount of sewage (with an average chlorine figure of about 10 parts per 100,000), discharging into a large volume of sea water (with a

chlorine figure which may vary from 1,600 to over 1,900 parts per 100,000), has not sufficient effect upon the chlorine figure of the sea water to furnish evidence of sufficient delicacy.

The oxidized nitrogen figure is even more serviceable than the chlorine as a clue to the previous animal contamination of *fresh waters;* for in fresh waters a little (say 1 per cent.) sewage contamination leads to a rapid appearance of nitrates; but when sewage effluent with already formed nitrates is added to a fresh water, there is generally an initial reduction of the nitrates (often lasting for two or three weeks), or the figure may remain practically stationary for the first few days, and then a rise set in until a constant figure is arrived at. But it is remarkable how often in fresh waters no evidence is to be obtained of the presence of the intermediate or nitrite stage of the development of nitrates; and, when appreciable, how faint the evidence often is. In polluted *sea water,* however, the evidence of oxidized nitrogen may not be available; for under ordinary conditions, up to a period of several weeks, no oxidized nitrogen may appear in sea water as the result of sewage contamination; but it ultimately appears in amounts which give very definite reactions by qualitative tests, and more especially is this true of nitrites. When sewage effluent already containing nitrates and nitrites is added to pure sea water the nitrates disappear in a day or two, though a trace of nitrites may persist for much longer. Therefore, as nitrites may be in evidence in polluted sea water where nitrates are not, they furnish the better evidence of sewage contamination. Thus oxidized nitrogen in sea water will indicate contamination which is either very recent or very remote; but its absence is no guarantee of freedom from such contamination in a dangerous form, and may indeed be even more significant of danger than its presence in sea water which is believed to have been *recently* contaminated.

Turning next to the free and albuminoid ammonia figures, which form such a useful indication of animal contamination in fresh waters. When small proportions (1 per cent.) of sewage are added to *fresh water,* there is to be noted an increase of ammonia—generally slight—for the first few days; then a reduction sets in, and after several days or several weeks (according to season and dosage) this evidence of contamination has disappeared. When *sea water* is similarly contaminated, a slight preliminary increase is to be noted for two or three weeks,

and then reduction generally sets in slowly. Therefore the free ammonia figure is a very valuable clue to contamination of sea water, forming invariably an item of evidence which, starting at the actual time of contamination, persists for several weeks.

When small proportions of sewage are added to *fresh water*, an increase in the albuminoid ammonia figure is to be noted, which often persists for several weeks, so that the figure may reach one several times greater than the original figure; then an irregular fall sets in through many weeks. But in *sea water* we find that the preliminary increase is less rapid, the original figure being generally found after two or three weeks, and later there is some reduction. When this figure in pure sea waters is compared with the free and saline ammonia figure, it is found that not only is it always a much higher figure, but that it is subject to far greater variations. With these more indefinite characteristics the figure does not lend itself as a basis for computing the lesser degrees of sewage contamination.

Pure sea water has a considerable reducing action upon potassium permanganate under the conditions of the processes employed in water analysis. The oxygen absorbed figure in pure sea water generally approximates to 0·5 part per 100,000 in four hours at the temperature of the laboratory, and careful analysis may furnish no appreciable difference when sea water is polluted with 1 per cent. of sewage effluent. It is obvious, therefore, that this process does not assist us in determining the presence of the lesser degrees of contamination of sea water.

Nor does the dissolved oxygen in slightly contaminated sea water furnish reliable results, for the figure falls with the time which has elapsed since the sample was taken; and the differences between pure sea water and sea water contaminated with 1 per cent. of sewage effluent are often so slight as to be unserviceable.

Phosphates are absent from pure sea water, and their presence is valuable corroborative evidence of contamination. The evidence, however, may be obscure where the contamination is slight; but working with 100 c.c. of sea water, the ammonium molybdate reaction is generally appreciable when sea water is contaminated with quite small proportions of sewage.

Interesting and suggestive as are the results of laboratory experiments extending over many weeks, one may not argue from the results of sewage contamination of stationary sea water employed in experiments to sea water moving under the in-

fluence of tides, eddies, and currents. To keep to the practical issues of the problem it is necessary to observe the behaviour of sewage contamination during a period of at most several days instead of several weeks; and it is upon these considerations that the following conclusions upon the chemical evidence of slight sewage pollution of sea water are based.

We may conclude that the free and saline ammonia figure furnishes the only reliable chemical guide to the lesser degrees of animal contamination of sea water; that delicate corroborative evidence may sometimes be obtained from the presence of nitrites and phosphates; but the complete absence of oxidized nitrogen is compatible with recent pollution, and it is not always easy to obtain a definite reaction for phosphates when the contamination is but slight. The free and saline ammonia figure remains, of all the available tests hitherto suggested, the most reliable and the most delicate; and an ammonia figure much exceeding 0·002 part per 100,000 is certain evidence of the sewage contamination of sea water.

SAMPLES OF PURE SEA WATER, OBTAINED FROM POINTS AT WHICH THE WATER WAS JUDGED TO BE FREE FROM POLLUTION (PARTS PER 100,000).

Neighbourhood where Sample collected.	Free and Saline Ammonia.	Albuminoid Ammonia.	Nitrogen as Nitrates and Nitrites.*
Aberdeen	0·001	0·007	0·00
Bournemouth	0·0018	0·002	0·00
Carnarvon	0·0015	0·006	0·00
Clacton	0·001	0·004	0·00
Folkestone	0·002	0·004	0·00
Hastings	0·0015	0·003	0·00
Ilfracombe	0·0015	0·0075	0·00
Oban	0·001	0·009	0·00
Scarborough	0·001	0·005	0·00
Ventnor	0·001	0·016	0·00

There is also available the method of taking several samples for comparative purposes and of judging the presence and degree of contamination in any particulars ample from any observed variations—more particularly, in the case of sea water, in the

* Professors E. A. Letts and E. H. Richards find that a trace of nitrates (averaging 0·005 N per 100,000) may be demonstrated in pure sea water by adding to 25 c.c. of the water a few drops of brucine sulphate solution and 2 c.c. of strong H_2SO_4.

ammonia and oxidized nitrogen figures. This method will often be serviceable, and should always be availed of whenever it is possible to obtain fair control samples from situations obviously more remote from any source of contamination.

In analyses of sea water, the nitrates should be tested for, qualitatively, by brucine and sulphuric acid, and the nitrites by Ilosvay's method. The oxidized nitrogen should be estimated quantitatively by the wet copper-zinc-copper process; the oxidizable organic matter by Tidy's process, conducted at the laboratory temperature; and the dissolved oxygen by Winkler's process. The phosphates should be tested in 100 c.c. of the sample by first adding strong nitric acid, and then evaporating to a solid residue; the residue to be then digested in strong nitric acid and the filtrate tested with molybdic solution.

CHAPTER XVI

ALKALIMETRY AND ACIDIMETRY—ICE—MINERAL WATERS —ANALYTICAL SCHEMES

ALKALIMETRY AND ACIDIMETRY.

FOR the purposes of estimating the degree of alkalinity or acidity of water, it is convenient to use standard solutions based upon the atomic or molecular weights of the different reagents, or made up so that equal volumes of the solutions are chemically equivalent to each other.

Such solutions are "normal" when they contain in 1 litre at 16° C. chemically equivalent weights of the active reagents weighed in grammes, hydrogen being taken as the unit. Therefore the normal solution of hydrochloric acid must contain the molecular weight of the acid—*i.e.*, 36·46, in grammes per litre, since HCl is a univalent substance. The normal solution of sodium carbonate (Na_2CO_3) must contain the molecular weight of the salt $\{(23 \times 2) + 12 + 16 \times 3)\}$ divided by $2 = 53$ grammes per litre; for the molecule of monobasic HCl can neutralize only half a molecule of the bivalent Na_2CO_3.

But the hydrogen equivalent of some reagents is not so easily arrived at. Take, for instance, potassium permanganate; the molecular weight of the formula ($K_2Mn_2O_8$) is 316, and the normal solution is 31·6 grammes per litre. This is because 316 grammes of permanganate of potash liberate 80 grammes of oxygen, which are chemically equivalent to 10 grammes of hydrogen; and so 31·6 grammes of the salt are equivalent to 1 gramme of hydrogen. $\left(\underset{(316)}{K_2Mn_2O_8} + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 3H_2O + \underset{(80)}{5O}.\right)$

"Seminormal" and "decinormal" solutions are obviously those made up to $\frac{1}{2}$ and $\frac{1}{10}$, respectively, of the strength of

the "normal" solutions. They are commonly expressed as $\frac{N}{1}$ and $\frac{N}{10}$ solutions.

Thus each c.c. of a normal solution of HCl will contain $\frac{1}{1000}$ of the molecular weight of the acid in grammes (*i.e.*, 0·03646 gramme) and each c.c. of a decinormal solution will contain 0·003646 gramme.

Therefore measured quantities of normal and decinormal acids should exactly neutralize similar quantities of the normal and decinormal alkalies; and if, on titration, they are not found to quite correspond, the difference must be ascertained and a simple calculation made in order to correct it.

In estimating alkalinity the decinormal solution of hydrochloric acid may conveniently be employed, and for acidity the decinormal solution of sodium carbonate. In either case one of these standard solutions would have to be added in measured quantity until the neutral stage is exactly reached, as indicated by a suitable reagent which is added to the solution.

Methyl-orange (about 1 gramme to the litre) is a good "indicator" where the alkalinity of water is being tested. This substance has the property of yielding a beautiful scarlet colour in the presence of acidity; but its solution, which is of a bright orange colour, must not be employed where organic acids are concerned or where nitrites are present. In these cases phenolphthalein may be substituted; but not if free carbonic acid is present. Phenolphthalein dissolved in 50 per cent. alcohol is a colourless solution which strikes a rose-red colour in the presence of alkalinity, but is colourless in acid solutions. The marked presence of ammonium salts would vitiate the results when phenolphthalein is employed. Litmus should not be used as an "indicator," for the CO_2, so commonly present in water, considerably masks its indications. A solution of cochineal is almost free from this drawback, and is a useful indicator; it is prepared by digesting the dried and powdered cochineal in warm water to which a little alcohol has been added, and then filtering; the solution has a yellow or yellowish-red colour, which is turned violet-red by alkalies, and the original colour is restored by mineral acids.

A 1 per cent. solution of rosolic acid in dilute alcohol is a delicate indicator. A rose colour forms in alkaline solutions, and even the presence of CO_2 or acid salts prevents this colour from forming.

Example.—It is desired to estimate the alkalinity of a water sample. A few drops of the methyl-orange "indicator" are added to 100 c.c. of the water in a white porcelain dish. A decinormal (or centinormal) solution of HCl is then dropped in from a graduated burette until evidence of a scarlet tint appears, denoting all alkalinity to be neutralized. It took 6 c.c. of the decinormal acid to effect neutrality; therefore the alkalinity is equivalent to 6 c.c. of this acid solution. But 6 c.c. of the decinormal HCl is equivalent to a similar amount of decinormal sodium carbonate solution; therefore the alkalinity is equivalent to 6 c.c. of decinormal sodium carbonate solution.

But 1 litre of the normal solution contains 53 grammes of sodium carbonate; therefore 1 litre of the decinormal solution contains 5·3 grammes, and 1 c.c. of this contains 0·0053 gramme, and 6 c.c. contain 0·0318 gramme of sodium carbonate.

Therefore the alkalinity of 100 c.c. of the solution is equivalent to 0·0318 gramme of sodium carbonate, or 31·8 parts of sodium carbonate per 100,000.

In estimating the acidity of a peaty water, the "indicator" —one or two drops of an alcoholic solution of phenolphthalein— is added, and the sodium carbonate decinormal solution run in until a very faint pink tint is obtained, when the calculation is made as above.

To prepare the normal HCl it is necessary to take about 181 grammes of liquid acid of the S.G. 1·10, and dilute to a litre with water; then titrate the exact strength with normal sodium carbonate.

ICE.

Both artificial and natural ice are liable to furnish on analysis considerable evidence of pollution, which, since pathogenic organisms can survive in ice for long periods, must be regarded as significant of danger. Under natural conditions the most superficial layer of the ice contains most impurity.

The popular belief that water purifies itself by freezing is unfounded. It is certainly not borne out when the water obtained from the melted ice is subjected to chemical analysis and bacteriological examination.

The results of many analyses performed in this country, America, and the Continent show the following variations:

	Parts per 100,000.
Free and saline ammonia	from 0·001 to 0·32
Albuminoid ammonia	,, 0·002 to 0·44
Chlorine as chlorides	,, 0·1 to 6·5
N as nitrates	,, nil to traces
Total solid matter	,, 1 to 50

Bacteria per c.c., 40 to 2,000.

Mineral Waters.

The examination of mineral waters for public health purposes should be conducted on precisely the same lines as that of an ordinary water analysis; that is to say, an effort must be made to ascertain the freedom of the water from dangerous organic and metallic contamination.

Artificial mineral "waters" consist of water into which carbonic acid is forced under pressure. Lithia water is, in addition, charged with lithia; potass water with bicarbonate of potash; soda water is commonly sold without the addition of any soda, but when such is added it is usually to the extent of about 10 grains of bicarbonate to the pint.

Natural mineral waters are generally the purest. Those which are chalybeate mostly contain the iron in the form of ferrous carbonate, held in solution by excess of carbonic acid, such as those at Tunbridge Wells, Spa, and Cheltenham. Instances of alkaline waters naturally charged with carbonic acid, and containing sodium carbonate and bicarbonate, are found at Carlsbad, Ems, Malvern, Nieder-Seltzers, and Vichy. At Harrogate and Aix-la-Chapelle waters are found naturally charged with sulphuretted hydrogen. Those waters which possess a marked aperient action generally owe their properties to either sulphate of magnesia, as at Epsom and Leamington, or to sulphate of soda, as at Cheltenham and Scarborough. In Central Wales there is a deep spring containing 9 parts of barium chloride per 100,000.

If the sample is collected from a well and it is desired to know the temperature of the water, the thermometer should be let down in a stout glass bottle; this will come up filled with the water

and a reading of the thermometer when surrounded by the water can be taken.

All artificial mineral waters should be tested for lead, iron, copper, zinc, and arsenic. Each of these metals has been found in samples of soda water, etc., to which the metal has gained access either by the apparatus used, the improper washing of the carbonic acid, or from the use of metal taps to the siphons. Sometimes a considerable quantity of lead is present and very impure water is used; on this account it is desirable that efficient supervision should be exercised over their manufacture.

Lemonade and ginger-beer are also liable to contain traces of lead, derived from the apparatus, and, in the case of the former, from the impure tartaric acid employed.

The sediment sometimes yielded by mineral waters after long storage generally consists of hydrated ferric oxide, alumina, silica, and calcium carbonate.

The carbonic acid of aerated waters is unfavourable to germ life, and the bacteriological counts are generally low.

In aerated waters the large amount of carbonic acid interferes with the estimation of the free and saline ammonia by Nesslerization, and must therefore be removed as follows: The ammonia should be fixed with 10 c.c. of normal sulphuric acid, then the water is heated to drive off the carbonic acid, and after neutralizing the acid with 10 c.c. of normal sodic hydrate, the ammonia may be distilled over and estimated.

When it is found necessary so to deal with carbonic acid, a blank experiment should be performed, in which any ammonia found in 500 c.c. of ammonia-free distilled water containing 10 c.c. of normal sulphuric acid and 10 c.c. of normal sodic hydrate is distilled over and estimated, and this is deducted in arriving at the figure of the free and saline ammonia in the sample.

Scheme for Effecting an Analysis in the Quickest and Most Convenient Manner.

Although a little confusion may be experienced at first, yet, after a little practice, the following plan will be found practical and expedient, the time required being about three hours.

1. Start the process for the estimation of the oxidizable organic matter.

2. Start the evaporation for the estimation of the total solids.

3. Start the concentration of the water for the purpose of testing for poisonous metals, etc.

4. Start the distillation for the estimation of the free and saline ammonia.

5. Start the water boiling for the estimation of temporary and permanent hardness.

6. Start the alkaline permanganate boiling in preparation for the second stage of Wanklyn's process.

7. Apply qualitative tests for nitrates, nitrites, sulphates, and phosphates.

8. Start the quantitative estimation of oxidized nitrogen (picric acid process).

9. Make the quantitative estimation of chlorine.

10. Make the quantitative estimation of total, temporary, and permanent hardness.

(By this stage the free and saline ammonia will be over, the alkaline permanganate may be added to the boiling-flask, and the distillation for the albuminoid ammonia started.)

11. Estimate the free ammonia.

12. Care has been taken throughout not to disturb any deposit which may be present. Now collect and make an examination of any sediment or suspended matter. Note the physical characters in the 2-feet tube.

13. Estimate the albuminoid ammonia.

14. Complete the picric acid process.

15. Estimate the oxygen absorbed.

16. Test for poisonous metals, and estimate quantitatively if any one is present.

17. Complete the estimation of the total, volatile, and non-volatile solids.

Scheme for the Detection of Poisons added to Water.

(2 litres required. Turbid waters should be regarded with special suspicion.)

1. To each of two porcelain bowls add 100 c.c. of the water. To one portion of the water add 2 drops of ammonia and ammonium sulphide. Start the evaporation of these waters to dryness over the water-bath.

2. Next start the concentration of 300 c.c. of the water, made alkaline with pure sodium carbonate, to 100 c.c.

3. Then to 100 c.c. of the sample placed in a white porcelain bowl add 2 or 3 drops of a freshly prepared solution of sodium sulphide. A brownish discoloration or precipitate will indicate the presence of either *iron, copper, lead,* or *mercury.* (An orange colour would indicate the presence of *antimony.*)

Divide the discoloured water into two parts. To one part add 2 drops of hydrochloric acid—if colour discharged, it is due to *iron.* To the other part add a few drops of a solution of potassium cyanide—if coloration discharged, *copper* is present.

If discoloration remains after the addition of both hydrochloric acid and potassium cyanide, *lead* or *mercury* may be present.

Confirmatory Tests.—Place 50 c.c. of the water in a narrow glass cylinder; add a few drops of acetic acid, followed by a little potassium chromate solution—a haze at the end of fifteen minutes confirms the presence of *lead.*

To another 50 c.c. of the water placed in a narrow glass cylinder add 2 or 3 drops of acetic acid, and 1 drop of potassium ferrocyanide solution; if on standing five minutes a blue colour forms, this will confirm the presence of *iron;* if a bronze colour, this will confirm the presence of *copper;* and if a white flocculent precipitate, this will indicate the presence of *zinc.*

Dissolve the brown precipitate in a few drops of weak aqua regalis (4 parts of hydrochloric acid and 3 parts of nitric acid); liberally add a saturated solution of acetate of soda, and then a 10 per cent. solution of iodide of potassium in drops—a red precipitate results from *mercury.*

4. To each of two glass cylinders add 100 c.c. of the sample. Then to one cylinder add 2 drops of dilute sulphuric acid—a haze or marked precipitate, in five minutes, will indicate the presence of *barium;* to the other cylinder add a few drops of calcium chloride solution and ammonia—a slight haze or marked precipitate would indicate the presence of *oxalic acid.*

If barium is present, a little potassium chromate solution added to some of the original water will produce a fine silky precipitate soluble in dilute nitric acid.

5. Place 500 c.c. of the sample into a separating flask; add 3 drops of nitric acid, and allow to stand for three minutes; make alkaline with plenty of sodium carbonate; shake well add 20 c.c. of chloroform; mix thoroughly; let stand for fifteen minutes until all chloroform separates out;* carefully draw off the chloroform into two dishes; evaporate to dryness over a water-bath; to residues add 2 c.c. of 10 per cent. sulphuric acid, and mix thoroughly with glass rod; then to one dish add a few drops of Bouchardat's reagent (a solution made up in the proportion of 12·7 grammes of iodine and 60 grammes of iodide of potassium, to 1 litre of water); to the other dish add a few drops of phosphotungstate of sodium solution—a brownish precipitate in the first case, and a flocculent white precipitate in the second, will indicate the presence of an *alkaloid*.

6. When the dishes of step 1 are dry, add to the dish to which ammonia and ammonium sulphide were added a little water, 2 or 3 drops of hydrochloric acid, and a few drops of ferric chloride solution—a red colour would result in the presence of a *cyanide*.

Add caustic soda and a few drops of ferrous sulphate to the original water; boil well; add hydrochloric acid until the water clears; let stand for thirty minutes—a blue colour would confirm the presence of a cyanide.

To the other dish of step 1 add 20 drops of strong sulphuric acid; mix with a glass rod; add 1 drop of potassium chromate solution—a purple colour would result from the presence of *strychnine*.

7. Test the concentrated water of step 2 for *arsenic* (Marsh's Test).

By the above scheme any one of the poisons mentioned would be detected if it were present in harmful amount.

* It is well to extract three times with chloroform.

PART II

SEWAGE AND SEWAGE EFFLUENTS

IN collecting samples of sewage or sewage effluents for analysis the average sample should be obtained in each case. This may be done by mixing together the hourly samples taken throughout the day, and these should vary in bulk in proportion to the flow of sewage or effluent at the time when each sample is taken; for instance, using bottles of one size for collecting all the samples, the bottle is filled if the maximum flow of sewage is observed at the time the sample is taken; if half the maximum, the bottle is half filled, and so on. When all these samples are mixed together, the mixture will fairly represent the average composition; and a part of this should be taken for the analysis. The composite sample should amount to at least a gallon.

It is of the greatest importance that the analysis should be performed as soon after the collection of the sample as possible, for important changes may be rapidly brought about by the teeming micro-organisms present. About $\frac{1}{3}$ litre is the amount required for a complete analysis. The bottle should be quite filled with the sample to be analyzed, and if more than a day passes before the analysis is undertaken it should be kept in a cold chamber in the interval.

The estimations made in the analysis of sewage and sewage effluents include: The free and saline ammonia, the albuminoid ammonia, the chlorine, the oxidized nitrogen, the total solids in solution (volatile and non-volatile), and the suspended matter. The estimation of the dissolved oxygen absorbed by an effluent is displacing that of the oxidizable organic matter which was formerly so generally employed. In addition, so far as effluents are concerned, the physical characters are noted, and not infrequently an incubation test (at about 27° C.) is applied, in order

to see if the sample develops odour at the end of a day or two. As a general rule, a sample of effluent is allowed to deposit before the quantities are removed for analysis; and as in samples of sewage the matter remaining in suspension is considerable and leads to variable analytical results, two or more analyses should be performed of the sample, and the mean taken of the different figures obtained; and the solids may be estimated both before and after the sample has been shaken, and the two results given.

The results of the analysis should be expressed in terms of parts per 100,000 to the second place of decimals; analyses which record a third or fourth place of decimals give a fictitious appearance of accuracy when such a changeable substance as sewage or as sewage effluent is concerned.

The analysis proceeds upon similar lines to those of water analysis, but it is necessary to dilute the sewage matter to a considerable extent before commencing certain of the estimations. So far as the solids are concerned, these may be estimated from the original sewage or effluent, but for the calculation of the two ammonias 20 c.c. of *sewage effluent* and 10 c.c. of *sewage* should be made up to the litre with ammonia-free distilled water, and the results obtained will represent the amounts in 20 c.c. and 10 c.c. respectively. In Nesslerizing the ammonias the contents of the Nessler glasses may be all mixed together in a beaker, and the colour of 50 c.c. matched; thus, supposing 200 c.c. of the distillate were collected before all the free "ammonia" had come over, then the ammonia estimate in 50 c.c. must be multiplied by 4. For Tidy's process 20 c.c. of effluent and 10 c.c. of sewage should be added to 100 c.c. of distilled water. The nitrates may be estimated from the original effluent by the phenol-sulphonic acid method. For the estimation of the chlorine the effluent should generally be first diluted with an equal quantity of distilled water. The amount of chlorine is practically unaffected by the usual methods of sewage purification, and if its quantity is not added to by the waste liquors from manufacturing processes (ferrous chloride—iron pickle, or salt) it furnishes a useful clue to the strength of the original sewage. About 10 parts per 100,000 may be taken to indicate sewage of average strength. It is generally necessary to filter the sewage, and occasionally the effluent, before making this quantitative estimation.

In performing Tidy's process it will often be noted that the solution becomes decolorized at the bottom first. The flask

should, therefore, be shaken from time to time. Various coal-tar products, indigo, logwood, and other dyes, and such salts as thiocyanates, sulphites, and sulphides, will also absorb oxygen in addition to oxidizable organic matter.

In performing Wanklyn's process, if the ammonias come over very slowly, so that the water in the boiling-flask reaches below 150 c.c., then a further 100 c.c. of ammonia-free distilled water should be added to the boiling-flask. In the case of sewage or bad effluents it is sometimes difficult to get all the albuminoid ammonia over without adopting this expedient.

In the estimation of N as nitrates the picric acid process is to be preferred, but this method may yield unsatisfactory results if the effluent contains waste gas liquors. In such a case the effluent should be diluted, and the wet copper-zinc couple method employed.

The amount of suspended matter will indicate the amount of deposit likely to take place in a stream. It may be estimated with sufficient accuracy by collecting it from 500 c.c. of effluent or 100 c.c. of sewage, on previously well-washed, dried, and weighed fine, hard filter-papers.

The albuminoid ammonia figure is a fair indication of the amount of organic matter, but the organic nitrogen, as estimated on Kjeldahl's principle, is a much more inclusive estimation than the albuminoid ammonia, and it is almost as easily arrived at. Although it will be found that the organic nitrogen of Kjeldahl's process averages a little over twice the nitrogen of the albuminoid ammonia (though sometimes showing marked departures from this average), the fact that the two analytical figures do not bear a constant ratio to one another is significant, and points to the desirability of adopting the more inclusive estimation.

The presence of oxidized nitrogen in an effluent must not be regarded as insuring absence of odour, although if nitrates are found to persist in an inoffensive effluent for a few days after its collection, the effluent is not likely to become offensive. Nitrates are a measure not of that pollution which may be oxidized, but of that which has been oxidized, and their presence often gives little indication of what remains to be purified; but generally high nitrates are a good feature in a sewage effluent.

ESTIMATION OF ORGANIC NITROGEN BY KJELDAHL'S METHOD.

1. Place 20 c.c. of the sewage or sewage effluent into a small flask, and after adding about 1 c.c. of sulphuric acid evaporate over the water-bath to about 5 c.c.

2. Add 20 c.c. of pure concentrated H_2SO_4, close the mouth of the flask by a small glass funnel, and boil slowly for an hour or two until the solution is of a clear, pale yellow colour.

3. Let cool, and then transfer the contents of the small flask to a distilling flask, being careful to well wash out the small flask and to transfer the washing to the distilling flask.

4. Make up the bulk of liquid to about 500 c.c. with ammonia-free distilled water, and then neutralize the acid with excess of strong potassic hydrate solution. The amount necessary to add can be determined by previously ascertaining how much is required to neutralize, say, 22 c.c. of the concentrated acid in water.

5. Heat a piece of pumice-stone to bright redness, and drop it into the flask to prevent "bumping." Distil over about 400 c.c., receiving the first portion of the distillate into a flask containing 20 c.c. of ammonia-free water slightly acidulated with two drops of dilute sulphuric acid, the distillate being received direct into this solution.

6. Nesslerize the ammonia; and $\frac{14}{17}$ of this will be nitrogen.

7. Deduct the amount of nitrogen as "free and saline ammonia" in 20 c.c. of the original sewage or effluent (previously ascertained by Wanklyn's process), and the difference = the organic nitrogen. Calculate to parts per 100,000.

8. Always make a blank experiment to determine the amount of ammonia thus obtained from the reagents and distilled water employed, and deduct this amount.

Note.—By the action of the sulphuric acid the nitrogen of the organic matter is converted into sulphate of ammonia. The potassic hydrate liberates the ammonia, which is then distilled over.

The above is a simple method of estimating the organic nitrogen, giving results which vary but little from those obtained from working with the solid matter of a litre of water. In the latter case a few grains of yellow mercuric oxide and of potassium sulphate should be added to the solids along with the sulphuric

acid, and, before distillation, 50 c.c. of potassium sulphide solution (40 grammes to the litre) should also be added.

The composition of sewage from the same district varies very greatly from time to time, and this is true, though to a less extent, of the effluent. The effluent, moreover, varies according to the method and degree of treatment; but the following mean results of many analyses will serve to give the reader a general idea of the composition of domestic sewage and sewage effluents:

	Sewage as it leaves the Outfall Sewer. Parts per 100,000	Effluent after the Sewage has been chemically treated and then passed through Filter-beds. Parts per 100,000.
Free and saline ammonia	6·5	1·50
Organic ammonia	1·4	0·14
O absorbed in two hours at 27° C.	9·6	1·35
Nitrogen as nitrates and nitrites	0·0	1·20
Chlorine	11·1	10·5
Suspended matter	50	2·0
Solids in solution	90	75
(*a*) Volatile	50	32
(*b*) Non-volatile	40	43

Often it is desired to obtain an expression of the amount of purification obtained by a given method of treatment; it is then usual to calculate the percentage amounts of purification effected from the differences between the "albuminoid ammonia" and "oxygen absorbed" figures of the original sewage and those of the effluent. For this purpose the figures of the original sewage are taken as 100.

Example.—The albuminoid ammonia of the original sewage= 0·8 part per 100,000, and that of the purified effluent=0·2. The percentage purification would therefore be $\frac{100 \times (0{\cdot}8-0{\cdot}2)}{0{\cdot}8}=75$.

STANDARDS OF PURITY OF SEWAGE EFFLUENTS.

A satisfactory sewage effluent must be without fæcal odour, and should possess little colour or turbidity. It has been suggested that pearl type should be readable through a column of effluent 10 inches in depth. In parts per 100,000 the figure of albuminoid ammonia should not exceed 0·1 or 0·15, nor the oxygen absorbed by oxidizable organic matter in two hours at 27° C. a figure of 1·5. The final effluent must not be liable to

putrefaction or secondary decomposition; and, if satisfactory, all frothing will disappear in three seconds after a half-filled bottle if shaken vigorously for one minute.

The Royal Commission on Sewage Disposal, appointed in 1898, found as follows:

The harm caused by allowing unpurified, or imperfectly purified, sewage to flow into rivers and streams may be placed under one or more of the following headings: The de-aeration of the water of the river, and consequent injury to fish; the putrefaction of organic matter in the river to such an extent as to cause nuisance; the production of sewage fungus and other objectionable growths; the deposition of suspended matter, and its accumulation in the river-bed or behind weirs, which will draw upon the oxygen in the supernatant water; the discharge into the river of substances, in solution or suspension, which are poisonous to fish or to live-stock drinking from the stream; the discoloration of the river; and the discharge into the river of micro-organisms of intestinal derivation, some of which are of a kind liable, under certain circumstances, to give rise to disease. The extent to which the purification of a sewage need be carried varies with the particular circumstances of the town and river concerned, and they recommend that local circumstances should be taken into account. The effect of an effluent on a stream does not generally depend on the absolute amount of organic matter contained in it, but rather on the nature and condition of that organic matter, and the important thing to ascertain in examining an effluent is the extent to which the contained organic matter has undergone fermentation.

In the Eighth Report of the Royal Commission on Sewage Disposal the Commissioners deal with the question of the standards to be applied to sewage and sewage effluents discharging into rivers and streams, and the test which, in their opinion, should be used in determining those standards. The Commissioners reiterate a previous recommendation that Local Authorities should not be required to purify their sewage more highly than is necessary to obviate the risk of actual nuisance (from odour, growths, putrefying solids, and detriment to fish life) arising from its discharge. They express the view that an effluent ought not to be considered alone, but that the nature and volume of the recipient waters should always be taken into consideration; and that any standard laid down may be either a

general standard or a special standard which will be higher or lower than the general standard, as local circumstances require or permit. They find that the nuisance-producing power of an effluent is broadly proportional to its deoxygenating power on the stream; and it is recommended that an effluent, in order to comply with the general standard, should not be permitted to contain more than 3 parts per 100,000 of suspended matter, nor with the suspended matters included should the effluent take up more than 2 parts per 100,000 of dissolved oxygen in five days when it is maintained at a temperature of 18° C.

The Commissioners classify rivers as follows:

"Very clean" if no more than 0·1 part of oxygen is absorbed.
"Clean" if no more than 0·2 part of oxygen is absorbed.
"Fairly clean" if no more than 0·3 part of oxygen is absorbed.
"Doubtful" if no more than 0·5 part of oxygen is absorbed.
"Bad" if more than 1·0 part of oxygen is absorbed.

The above figures represent the parts of dissolved oxygen taken up by 100,000 parts of the water in five days at 18° C.

"If 100,000 c.c. of river water (containing effluent) do not take up more than 0·4 gramme of dissolved oxygen in five days, the river will ordinarily be free from signs of pollution, but above this figure it will almost certainly show them"; and "if the mixture should yield a figure exceeding 0·4, then the effluent would have to be improved." "This figure (0·4) we term the 'limiting' figure, and in our opinion it should be the foundation upon which any scheme or standard should be constructed in order to ascertain the minimum degree of purification which would be sufficient to obviate risk of nuisance."

The albuminoid ammonia and acid permanganate tests only yield empirical results which do not sufficiently indicate the consequences which follow when effluents are discharged into streams; acid permanganate is too vigorous an oxidizing agent; and the amount of dissolved oxygen taken up from water by an effluent is doubtless a test which possesses an advantage as an indication of what naturally takes place.

In the experience of the Commissioners, if the dilution of the effluent, while not falling below 150 volumes, does not exceed 300, the dissolved oxygen absorption test may be omitted, and the standard for suspended solids fixed at 6 parts per 100,000.

When the dilution, while not falling below 300 volumes, does not exceed 500, the standard for suspended solids may be further relaxed to 15 parts per 100,000. Lastly, with a dilution of over 500 volumes, the Commissioners conclude that all tests may be dispensed with and crude sewage discharged, subject to such conditions as to the provision of screens or detritus tanks as may appear necessary to a Central Authority. It is not possible, nor is it necessary, to lay down fixed standards with reference to tidal waters.

Some effluents, especially those containing iron salts, while free from visible suspended solids at the time of sampling, are capable of yielding considerable deposits on standing. Therefore, as suspended matter is liable to separate out of true and

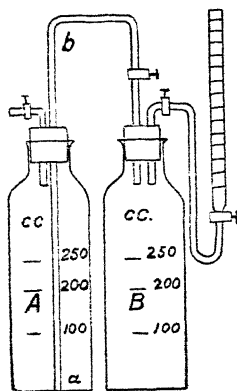



FIG. 17.—APPARATUS FOR ADENEY'S PROCESS.

colloidal solution on standing, any delay in making the estimation is important. Furthermore, by delay, the suspended matter may lead to a material reduction of the oxygen in the effluent.

To determine the amount of oxygen which a sewage effluent will absorb in a given time, a definite quantity of the effluent is allowed to remain in contact either with atmospheric air or with water containing a high figure of dissolved oxygen. For the former test the following method is satisfactory:

Adeney's Method for the Determination of the Rate of Absorption of Dissolved Oxygen in Polluted Waters.—A known volume of the effluent or polluted water—100 to 250 c.c., according to its strength—is decanted into the bottle B, into which a little freshly precipitated magnesium hydrate has been previously added for

the purpose of fixing the carbonic acid in the water. A similar volume of the distilled water is poured into the bottle A. Similar volumes of air will thus have been left in the two bottles. The corks with the connecting tube and stopcocks in position are then fitted into their respective bottles, care being taken previously to open both stopcocks. A slight rise of water from capillary action will of course occur in the portion of the connecting tube in the bottle A; the height to which it rises in this way should have been previously marked by a writing diamond. This mark serves as an index for subsequent measurement. The two bottles so connected, and both stopcocks being still open, are completely immersed in the water-bath for a few minutes to allow of their contents assuming a common temperature. Both stopcocks are then closed, and at the same time the temperature of the bath and the height of the barometer are noted. The apparatus is taken out of the water-bath and dried, especially the corks, which when completely dry are coated with shellac varnish to prevent diffusion of air through them. It is then put into a mechanical shaker, by means of which the contents of the two bottles are kept in gentle motion.

As oxygen is absorbed by the polluted water from the atmosphere in B, the pressure of the atmosphere will be reduced relatively to that of the atmosphere in A, which will be unaffected by the distilled water. Consequently, the water from A will rise in the connecting tube *b* in proportion to the volume of oxygen absorbed by the polluted water from the atmosphere in B. The volume of oxygen which is indicated by the rise of the water in the connecting tube can be measured at any time by attaching, by means of a flexible tube, a burette containing distilled water at the temperature of the laboratory. As the water from the burette is cautiously allowed to flow into B, the water in the connecting tube will gradually sink back to the index, when the stopcock to the bottle B is closed; and the volume of water which has flowed from the burette is equal to the volume of oxygen which has been absorbed from the atmosphere of B at the temperature and pressure of the atmosphere obtaining at the commencement of the experiment. It may here be noted that the distilled water bottle A acts as a reference pressure bottle.

If a comparatively rapid absorption of oxygen occurs during the first hour or two, and this is followed by a slower and regular absorption, it may safely be taken to be due to the polluted water

being de-aerated to start with, and possibly also to the presence of easily and directly oxidizable substances in it, the subsequent slower and regular absorption being due to indirect oxidation accompanying the fermentation of the polluting matters.

This apparatus may also be employed for determining the strength of a crude sewage, or of any other polluted water in the crude state, after the solids in suspension have been separated. The sewage should, however, be diluted with four to nine volumes of tap water.

The errors affecting the estimation are negligible, provided care be taken at the commencement that the water and air in the two bottles are at a common temperature and that the apparatus is airtight in all parts.

Under many conditions of work it will be found preferable to employ some of the polluted water to be examined instead of distilled water for use in the reference pressure bottle A, after sterilizing it by the addition of a few drops of a concentrated solution of mercuric chloride and shaking it with air in order to thoroughly aerate it.

It is sometimes advantageous that the bottles employed should be larger than those indicated in the diagram, and should be of about 1,000 c.c. capacity.

The Dissolved Oxygen absorbed in Five Days.

The method adopted by the Royal Commission for this determination is that of Winkler, as modified by Rideal and Stewart, the particulars of which are as follows:

Reagents required :

1. Concentrated sulphuric acid.
2. Concentrated hydrochloric acid (free from chlorine).
3. $\frac{N}{8}$ permanganate (3·94 grammes $KMNO_4$ per litre).
4. Potassium oxalate (2 per cent. of the crystallized salt).
5. Manganous chloride (33 per cent. of the crystallized salt).
6. A mixed solution of caustic potash and iodide of potassium, containing 70 grammes KOH and 10 grammes KI per 100 c.c.
7. Sodium thiosulphate solution, containing 12 grammes of the salt per litre (keep in dark bottle).

The Process.

1. Well shake the effluent, in order to bring its dissolved oxygen content to something near that of the diluting water; wait a few minutes, and then measure 300 c.c. and gently mix with four times its volume of well-aerated tap water at 18° C. (This water

will contain in solution about 7 c.c. of oxygen per litre, or 1 part by weight of oxygen per 100,000 of water.)

2. Quietly and quickly fill four small bottles (capacity 340 or 360 c.c.) with this mixture, the bottles being allowed to stand full to the mouth for five minutes, and then stoppered.

3. Place two of the bottles in an incubator at 18° C. for five days.

4. Determine at once the dissolved oxygen in the water of the other two bottles, as follows:

(*a*) First add 0·9 c.c. of sulphuric acid and then sufficient of the permanganate to still provide a pink colour after twenty minutes. 1 to 2 c.c. of $\frac{N}{8}$ permanganate are generally sufficient for this purpose. Mix the contents, and let stand for twenty minutes.

(*b*) Remove the excess of permanganate by the addition of about 1 c c. of the oxalate solution; restopper and mix.

(*c*) When the liquid has become colourless,* 1 c.c. of manganous chloride solution is run into the bottom of the bottle, followed immediately afterward by 4 c c of the solution containing potassium hydrate and potassium iodide.

(*d*) Insert stopper, and turn over the bottle once or twice; let stand for a few minutes; again turn over once or twice, and then allow the hydroxides of manganese to settle.

(*e*) Add 5 c c. of the hydrochloric acid, restopper and let bottle stand in shade for five to ten minutes, with occasional rotations.

(*f*) Twenty c.c. of the liquid are now pipetted out and rejected, and the remainder is titrated with thiosulphate, as described on p. 88.

(*g*) The second bottle is treated in the same way, and the mean of the two results is taken.

(*h*) At the end of five days the dissolved oxygen in the incubated bottles is determined, and the mean of the two results is subtracted from the first mean. The difference multiplied by 5 gives the amount of dissolved oxygen absorbed by 100,000 parts of the effluent in five days.

Example : The dissolved oxygen in the mixture at the start. The capacity of the bottle=341 c.c.; subtract 20 c.c.; and volume of mixture tested=321 c.c. The thiosulphate used=6·95 c.c.; but each c.c.=0·3773 milligramme oxygen; ∴ 6·95 c.c.=0·3773 × 6·95 milligrammes of oxygen; and the dissolved oxygen in parts per 100,000 of mixture $=\frac{0{\cdot}3775 \times 6{\cdot}95 \times 100}{321}=0{\cdot}817$.

The dissolved oxygen in the mixture at the end of incubation similarly calculated=0·488 part per 100,000.

Thus 0·817−0·488=0·329 gramme of dissolved oxygen is

* In the case of poor effluents and tank liquors a brown precipitate may form, and this must be given time to disappear.

taken up by 100,000 parts of the mixture; and as $\frac{1}{5}$ of this mixture is effluent, $0{\cdot}329 \times 5 = 1{\cdot}65$ is the absorption of dissolved oxygen by 100,000 parts of effluent in five days at 18° C.

Notes.—It is recommended that all samples should be allowed to stand at 18° C. for forty-eight hours after sampling and before carrying out the test; and if it is not possible to commence the tests at the end of that time the sample should be meanwhile kept on ice.

For practical purposes no corrections are necessary for the few c.c. of added reagents.

The temperature of 18° C. (65° F.) represents the maximum temperature likely to be reached in river water. The temperature of the incubator should not vary more than one degree on either side of this standard temperature.

A few control determinations with tap water alone should be done from time to time, to make sure that the tap water itself does not take up any appreciable quantity of oxygen.

It has been found that the biological oxidation of a sewage effluent proceeds more slowly when the effluent is diluted with a hard tap water than when diluted with distilled water. The dissolved calcium salts (and iron when present) exercise an appreciable inhibitory effect on bacterial activity; and therefore rather than dilute the effluent with variable tap waters, as recommended by the Commissioners, it would be better to always employ distilled water.

Effluents conforming to a satisfactory standard may cause considerable growths of organic life, which may subsequently produce a nuisance. There is much to learn of the causation of these objectionable growths, but certain of them are doubtless promoted by the presence of nitrates. *Carchesium* (constituting whitish masses of filamentous growth, characterized by bell-shaped heads on thread-like stems), green growths of *Oscillatoria nigra* and *Spirogyra*, and coarser growths of water-weeds may grow in well-purified effluents, and imperfectly purified effluents may foster such grey growths as *Leptomitus* and *Sphærotilus*, or even *Beggiatoa*.

A large thin-leaf seaweed, of cabbage-green colour and known as sea-lettuce (*Ulva latissima*), like different species of the grass-like *Enteromorpha*, etc., flourishes in association with the sewage pollution of sea water. The ulva grows most extensively in those estuaries where the water is shallow and the tidal movements are

slow and ineffectual in carrying all the sewage pollution out to sea at each ebb of the tide. We are indebted to Professor E. H. Letts for much information with reference to this sewage seaweed. He finds that it absorbs nitrogen from the ammonia and nitrates of sewage origin, and that mussels attach themselves to it by their byssus threads. After the ulva reaches a certain size, wave action detaches most of it, and the small retained pieces are capable of continuing the growth of the plant. If the detached ulva is not swept out to sea, it accumulates on the shore, where, exposed to the sun and air, fermentative decomposition sets in, and a micro-organism reduces the sulphates (which are abundantly present in the tissues of the ulva) to sulphides; and eventually sulphuretted hydrogen is liberated and an intolerable nuisance results.

The weed is also found in some places where the sea water is free from sewage pollution, in sheltered and shallow waters with sluggish currents, and where the means for its anchorage exist.

In sewage-polluted mud the N could be determined in 10 grammes of the mud by the Kjeldahl method. Information may also be obtained as regards the deoxygenating qualities of the mud deposited in the bed of a stream. Twenty-five grammes of the mud should be mixed with 500 c.c. of tap water and 10 c.c. of the mixture (containing 0·5 gramme of the mud) made up to 100 c.c. with distilled water, when Tidy's permanganate process may be performed.

The dissolved oxygen absorption at 18° C. in twenty-four hours may be determined on 1 or 5 grammes of wet mud (according as the mud is foul or otherwise) by allowing the mud to remain in an airtight bottle in contact with a relatively large volume of water containing oxygen in solution; and Winkler's process, as modified by Rideal and Stewart, may be employed (pp. 150 and 151). A highly nitrogenous mud will usually take up much oxygen, and fine sulphide of iron undergoes oxidation very readily. The absorption of dissolved oxygen by polluted muds goes on for very long periods, but the twenty-four hours' test serves for *comparative* purposes; indeed, as there are no recognized standards, the presence and amount of contamination by animal matter are best proved by comparing the results obtained with those furnished by the mud collected at other parts of the river-bed which are obviously remote from possible contamination.

PART III

SOIL EXAMINATION

THE ANALYSIS OF SOILS

THE sanitarian will not often find it necessary to make a chemical analysis of soil; he may want to classify soils and to examine them for fæcal pollution; but generally for his purposes laboratory results are of very secondary importance to those of observations made upon the soil *in situ.*

Although the power of absorbing and retaining moisture is a consideration of the first importance from a health view—exercising as it does an important influence upon the health of whole communities—yet it is of little practical value to perform any tests in this connection upon small quantities of soil which are collected and brought to a laboratory. The amount of moisture retained is so largely dependent upon local factors that the most valuable information is always obtained by observations of the soil *in situ.* The amount of moisture in a sample of soil would be ascertained by drying 50 grammes on the water-bath, and then placing in the hot-air oven at 95° C. until the weight is constant.

The depth of the ground water and the extent of its fluctuations are often of great importance. The digging of trial-holes will enable the height of the ground water to be ascertained, and the fluctuations in the level of the ground water may be determined by some arrangement similar to that shown in Fig. 18, which sufficiently explains itself.

The method of testing the capacity which the soil possesses for holding water is obvious: The dried soil is weighed in a cylinder and then saturated with water (this may take hours in the case of clay); the water is allowed to drain off through very

fine muslin until no more drops fall, and the soil is then reweighed, the difference in the two weighings represents the weight of water the known weight of soil is capable of holding.

In **collecting samples** it must be borne in mind that the characters of the soil may vary within small areas and at different depths, so that many samples may have to be collected and analyzed before one can speak with accuracy of the composition of the soil of a small area. These samples may be taken by a long, narrow spade, or by means of an ordinary $1\frac{3}{4}$ to 2 inch auger screwed into the soil; and 2 or 3 kilogrammes must be collected

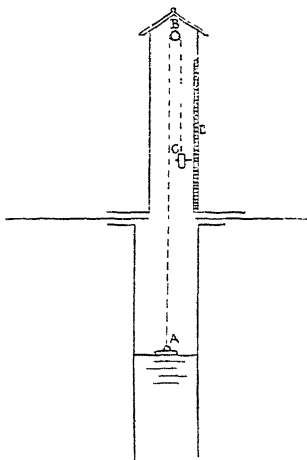



FIG. 18.—ARRANGEMENT FOR REGISTERING THE VARYING LEVELS OF THE GROUND WATER.

A, a float; B, a pulley; C, an index; D, a graduated scale. Opposite the scale D a narrow glass window is provided so that the scale can be seen without disturbing the arrangement.

for analysis. The depth of the surface soil varies considerably in different localities. In uncultivated grounds it generally occupies only a few inches in depth on the surface, and in cultivated grounds its depth is generally the same as that to which the implements used in cultivation have penetrated, which is generally from 9 to 12 inches.

Soil is composed of layers of more or less disintegrated rock and quantities of organic matter resulting from the decay of plants and animals. Entering into its composition are: The earths—silica, alumina, lime, and magnesia; the alkalies—soda,

potassa, and ammonia; the acids—sulphuric, hydrochloric, carbonic, nitric, phosphoric, silicic, and humic; oxide of iron and small portions of other metallic oxides; a considerable proportion of moisture (chiefly as a liquid film enveloping the particles); several gases, and micro-organisms. In addition to the so-called "nitrifying organisms," there are some which reduce nitrates to ammonia, and others that will fully oxidize ammonia in the presence of air, but will reduce nitrates to ammonia in the temporary exclusion of air. The variable amount of vegetable and animal matter gaining access is either partially or wholly decomposed, and is ultimately converted into water, carbonic acid, and nitric acid by the action of micro-organisms. Of the mineral matters, either silica, silicates, and double silicates, or calcium and magnesium carbonates, generally predominate. All soils contain, though in different proportions, the chief mineral constituents which are found in the ash of the plants which grow upon them; and an examination of such ash will often afford a rough-and-ready clue to the constitution of the soil. The colour of soil depends mainly upon the amount of humus, oxide of iron, and moisture. A dried soil is always much lighter in colour than when the moisture is present.

The less weathered stratum, which lies immediately under the soil, is called the **subsoil,** into the composition of which comparatively little organic matter enters. Sometimes this subsoil is porous sand or gravel; sometimes light and loamy and closely similar to the superimposed soil; sometimes stiff (clayey) and more or less impervious to water. The subsoil is generally lighter in colour than the soil, and its depth is usually limited by deposits of undecomposed or partly decomposed rock matter, or by deposits of clay, sand, or gravel.

The Classification of Soils.

The sample having been collected, the coarser stones should be removed, and all lumps broken up so far as possible with a wooden pestle.

The mechanical analysis of soils, or the sorting of the constituent particles into groups, is effected either by a stream of running water or by allowing the turbid mixture of soil and water to settle during varying periods of time, after the coarser particles have been removed and sorted by sieves of different-sized meshes.

Schloesing has insisted upon the necessity of first treating the soil with dilute acid and subsequently washing it, and of add ng ammonia to the water in which it is afterwards to be suspended. The acid dissolves calcium carbonate and decomposes "humates," and the liberated humic acid is dissolved by the ammonia. Otherwise the humates (if abundant) and the chalk tend to bind together the finest particles, which flocculate into loose aggregates which may not get disintegrated. The acid employed is too weak to dissolve any appreciable amount of mineral constituents other than calcium carbonate.

The groups of particles obtained in a mechanical analysis do not possess any definite chemical individuality. The coarser fractions may contain fine grains of quartz, particles of clay, ferric oxide, etc. It is likely that the physical properties of the soil depend rather on the size than on the chemical composition of the constituent particles.

Special apparatus has been devised, both for thoroughly crushing and also for washing and separating the various soil constituents seriatim. **Knopp's set of sieves** is useful for the purpose of classifying the coarse constituents of some soils. The soil is first dried, and then the lumps of soil are crushed up with the fingers and placed upon the top sieve with the coarsest meshes; no hard pestle must be used for the crushing, or mineral particles would be disintegrated or broken. After thorough shaking, the particles all separate out on one of six sieves, and the very fine material collects on a tray at the bottom of the apparatus. This latter material may be classified by means of elutriation, or washing.

Particles collecting on the top sieve are more than 7 millimetres in diameter, and=*coarse gravel;* those collected on the second, between 7 and 4 millimetres, and=*medium gravel;* on the third between 4 and 2 millimetres, and=*fine gravel;* on the fourth, between 2 and 1 millimetres, and=*coarse sand;* on the fifth, between 1 and 0·3 millimetre, and=*medium sand;* on the bottom, finer than 0·3 millimetre, and=*fine sand.*

What remains upon each sieve is weighed, and the results are expressed as percentages of the total weight.

Kuhn clasisfies everything coarser than 5 millimetres as *stones;* between 5 and 3 millimetres, as *coarse gravel;* between 3 and 2 millimetres, as *fine gravel;* between 2 and 1 millimetres, as

pearl sand; finer than 0·5 millimetre, as *fine sand;* and the portion separable by elutriation, as *earth.*

Elutriation may be performed by the washing cylinder of Knopp. This consists of a glass cylinder 55 centimetres high, to which are attached four glass tubes fitted with taps at intervals of 10 centimetres. The soil material which passes through a 0·3 millimetre sieve is placed in the cylinder, and this is filled with water to 10 centimetres above the highest tap. The whole is well shaken for five minutes, and then allowed to stand for another five minutes, when the top tap is opened and the cloudy water allowed to escape into a weighed dish. The material drawn off from the second and third and fourth taps is similarly

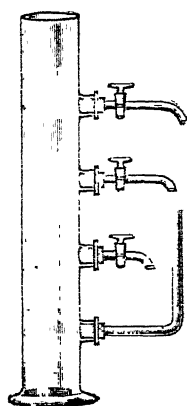



FIG. 19.—KNOPP'S SOIL-WASHING CYLINDER.

collected. The separate cloudy waters are then evaporated and the residues weighed; and the fine sandy residue at the bottom of the cylinder is also collected and weighed. In this manner the clayey matter in the fine soil can be further classified and compared with similar observations on other soils.

To constitute pure **clay** the particles should not exceed 0·01 millimetre, and the material should be previously treated with sufficient hydrochloric acid to dissolve out any carbonates; the washed soil should then be boiled for half an hour with 10 per cent. ammonia to dissolve humus, and the residue, washed, dried, and ignited, may be weighed as clay. This method is sufficiently exact for practical purposes.

Poquillon advocates the following method of estimating clay·

Ten grammes of the soil are rubbed up with 25 c.c. of water, and the liquid mixed with 100 to 120 c.c. of a 0·1 per cent. solution of ammonium chloride and left for five minutes. The supernatant liquid is then decanted; the operation is repeated six to eight times until the washings are clear, when the residual sand is washed, first with dilute hydrochloric acid and then with water, dried, and weighed. The turbid washings are mixed, acidified with hydrochloric acid, left for two or three hours, when the precipitated clay is collected on a filter, washed with water, dried, and weighed.

The amount of **sand in clay** is usually estimated as follows:

Heat a weighed quantity of the dried fine material with sulphuric acid; then boil with water; collect the insoluble matter on a tared filter, dry and weigh; remove and boil an aliquot part of this insoluble matter with a strong solution of sodium carbonate, and weigh the insoluble residue as sand.

Lime may be estimated by treating the earth in a litre shaking-flask for half an hour with 500 c.c. of standardized hydrochloric acid (containing about ½ per cent. of hydrogen chloride), and titrating an aliquot part with soda, using phenolphthalein as indicator.

The simplest apparatus for a **silt estimation** takes advantage of the relative rates of descent of the various soil particles through water at rest. The chief disadvantage of silt methods is the tendency of the fine particles to aggregate and form small lumps which act as larger particles. The only means of obtaining a perfect separation of the soil particles are by boiling for one hour with water, and by wet pestling. Even then it has been demonstrated that particles of the different sizes are represented throughout the entire deposit. Doubtless the finest particles are best separated by elutriation.

Any soil containing less than 5 per cent. of chalk, which is not so rich in vegetable matter as to constitute a "peaty" one, and which contains not over 10 per cent. of clay and excess of sand=a "*sandy soil.*" If such soil contains 10 to 40 per cent. of clay and excess of sand=a "*sandy loam.*" If 40 to 70 per cent. of clay=a "*loamy soil.*" If 70 to 85 per cent. of clay=a "*clay loam.*" If 85 to 90 per cent. of clay=a "*strong clay soil.*"

Sand makes a soil friable, gives it a low specific heat and the power of draining quickly.

A clay soil containing no sand at all=a "*pure agricultural*

clay," which is essentially silicate of alumina mixed with small quantities of organic matter, lime, magnesia, and ferric oxide. The different varieties of clay are mainly due to the varying amounts of these latter substances.

Strong clays absorb and retain nearly three times as much water as sandy soils, while peaty ones absorb a still larger proportion; and the same remarks broadly apply to the relative readiness with which water is lost by evaporation from these soils.

If there is more than 5 per cent. of chalk, the remainder consisting mainly of clay, the soil is called a "*marl*"; and if there is more than 20 per cent. of chalk, "*calcareous*."

"*Peaty*" soils generally contain from 60 to 80 per cent. by weight of organic matter; "*rich cultivated soils*," from about 5 to 20 per cent.; and "*stiff clayey*" ones, from 2 to 10 per cent.

By means of vegetation, and owing to the fixation of free nitrogen by soil micro-organisms and plants, even a sandy soil may in time become productive.

To ascertain **the substances which a water will extract** from soil and hold in solution, Schulze's method is recommended by Fresenius. The necks of several middle-sized funnels are closed with small filters of strong filter-paper; these are moistened, and the paper pressed close to the sides of the funnel; the air-dried soil is then introduced in small lumps ranging in size from a pea to a walnut (but not pulverized, or even crushed) until the funnels are filled to about two-thirds. Distilled water is now poured on in quantity sufficient to cover the soil. If the first portion of the filtrate is turbid, it must be poured back into the funnel, and the filtration allowed to proceed quietly; the funnels are again filled with water, and this process of extraction is continued until the combined filtrates weigh twice or three times as much as the soil used. The several filtrates are next mixed, and the necessary analysis performed to obtain the desired information.

Alumina was never found by Schulze in the aqueous extract.

In most soils the phosphoric acid exists as a basic ferric phosphate, and hence the great insolubility of soil phosphates.

The smaller part into which the non-concentrated aqueous solution was divided is finally tested for nitric and nitrous acids and ammonia.

As, however, the solvents which act naturally on the soil are something more than distilled water, it is desirable to examine those substances which are soluble in carbonic acid water, as by saturating distilled water with carbonic acid and allowing this to act upon the soil for several days in a closed flask, which should be well shaken from time to time. Water containing both carbonic acid and ammonium chloride (about 0·05 per cent.) should also be allowed in a similar manner to act upon the soil, and the substances *then* taken up should be examined.

Probably the best solvent for extracting from soil the "available" (as distinguished from "total") mineral constituents for plant food is a 1 per cent. solution of citric acid (Bernard Dyer).

The total **phosphoric acid** in soils should be determined in the manner recommended by Hehner, as follows: The soil is incinerated and digested with hydrochloric acid, evaporated to dryness to render silica insoluble, redigested with acid, filtered and washed. The filtrate and washings are concentrated to a small bulk, and treated in the cold with excess of a solution of ammonium molybdate in nitric acid. After standing forty hours in a warm place, the liquor is decanted through a filter, the precipitate is washed several times by decantation (first with dilute nitric acid, then with very small amounts of distilled water), and finally transferred to the filter and washed free from excess of acid. The ammonium phospho-molybdate is then dissolved in ammonia, evaporated to dryness in a platinum capsule, and dried to constant weight at 100° C. The residue contains 3½ per cent. of its weight of phosphoric acid. Or the ammonium phospho-molybdate may be dissolved in ammonia, magnesium mixture added, and the precipitate collected, ignited, and weighed as $Mg_2P_2O_7$, which $\times\ 0{\cdot}64 = P_2O_5$.

To estimate **nitric acid,** first rapidly dry the sample at about 60° C., so as to stop nitrification ensuing after the collection of the sample; extract 1,000 grammes of fine soil with 2,000 grammes of distilled water for forty-eight hours, with frequent shaking; and then filter 1,000 c.c. of the extract (corresponding to 500 grammes of the dry soil). A small quantity of pure sodium carbonate should be added to the filtrate, which is next evaporated to about 100 c.c. Any precipitate which forms during evaporation should be filtered off, when the nitric acid may be estimated in the filtrate.

Sulphur exists in soil as **sulphates** (generally calcium sulphate),

in organic compounds, and as sulphides (iron pyrites). To estimate the sulphates in soil, heat along with dilute hydrochloric acid for a short time, then filter, and precipitate from the filtrate with barium chloride solution.

An examination for the **peaty acids** may be made thus: Some of the washed soil is dried and sifted, to separate any straw, roots, and stones; what passes through a fine sieve is digested for several hours at about 30° C. with a solution of carbonate of soda, and filtered. The filtrate is then slightly acidified with hydrochloric acid; and if brown flakes separate, these consist of the peaty acids—*i.e.*, ulmic, humic, or geic. The more ulmic acid is present the lighter is the shade of brown; a dark shade indicates a preponderance of humic or geic acids.

These flakes may be collected upon a weighed filter, washed until the water begins to be coloured, dried, and weighed. Then burn the dry mass, deduct the weight of the ash (after subtracting the filter ash) from that of the dry mass, and enter the difference as "acids of humus."

The total nitrogen of soil would be best determined by Kjeldahl's process:

Five to ten grammes of the fine air-dried soil are placed into a small hard Jena glass flask, and 30 c.c. of pure sulphuric acid are poured over the soil, so as to thoroughly wet it. When all the frothing has subsided, 15 grammes of potassic sulphate are added (to raise the boiling-point), and about $\frac{1}{2}$ gramme of colourless (anhydrous) cupric sulphate (as an oxidizer), and the mixture is heated until the liquid is a yellow colour. Then 50 per cent. caustic potash solution (recently boiled to expel any ammonia) are added until the liquid becomes alkaline, as indicated by the circumstance that the copper is precipitated as blue cupric hydroxide. The remainder of the process is carried out in the manner described on p. 144.

Example.—10·55 grammes of soil were taken.

The distillate received into 50 c.c. $\frac{N}{10}$ sulphuric acid required 27 c.c. $\frac{N}{10}$ sodic hydrate to neutralize it.

∴ 23 c.c. of the $\frac{N}{10}$ acid have been neutralized by the ammonia in the distillate.

But 1 c.c. of the $\frac{N}{10}$ acid=0·0017 gramme NH_3 or 0·0014 gramme of N.

∴ there are 23 × 0·0014=0·0322 gramme N in 10·55 grammes of soil=0·3 per cent.

The collection of ground air and the estimation of carbonic acid are dealt with in Air Analysis.

It is occasionally desirable to know whether the soil has been recently polluted with excremental matter. The filtered aqueous extract (obtained by acting upon a known weight of dried soil with distilled water for forty-eight hours, with frequent stirring). can in these cases be examined for oxidized nitrogen, chlorine, and organic matter, and the amounts thus obtained compared with those procured from similar soil in the neighbourhood.

In 100 parts of soil dried in the air Krocker found that clayey soils, before manuring, yielded 0·1 to 0·45 of ammonia; loamy soils, 0·13; sandy soils (never cultivated), about 0·05; and marls, 0·004 to 0·09 of ammonia.

Ferrous sulphide is always in evidence in foul sewage deposits and in mud exposed to gross sewage contamination. Its presence has been explained by fermenting organic matter reducing ferric oxide or hydrate to ferrous compounds; then some of the sulphuretted hydrogen from the decomposition of organic matter forms the ferrous sulphide; and carbonic acid, acting on ferrous sulphide, is capable of producing the sulphuretted hydrogen which may cause an offensive nuisance (*vide* pp. 118 and 153).

As would be inferred, the **soil of graveyards** above the burial level does not materially differ, as regards the amount of organic matter and its products, from similar soil (unmanured) elsewhere; but that taken on the level of the coffins, and from a short distance below, is relatively richer in organic matter. Such soil is found to be somewhat richer in bacteria than other unmanured soils, and more especially is this the case with that lying around the top of the coffins (Reiners, Fraenkel, Young).

The various **manures** with which the soils under cultivation are dressed necessarily effect considerable changes in the constitution of the original soil, besides yielding abundance of soluble matter to the water which comes in contact with them. The commoner manures are—

Farmyard and animal excrement and "guano"; bone dust and other phosphatic manures (calcium phosphate), etc.; vegetable manures—sawdust, soot, charcoal, peat, and seaweed; ammonia salts, especially the sulphate; sodium salts, especially the nitrate; potassium salts, especially the chloride, nitrate and phosphate; and gypsum.

The following are some of the recommendations of a Committee of the Agricultural Education Association:

Taking Samples.—Under ordinary conditions the sample shall be taken to a depth of 9 inches, but in case of shallow soils to such lesser depths as mark a natural line of demarcation.

The committee approves of the use of the auger as one method that may be adopted for taking samples. Several cores should be taken and mixed for analysis.

Drying.—The sample shall be air-dried for analysis. The drying may be accelerated by heating to a temperature not exceeding 40° C., but in all cases the soil should be finally left, for a day or two, spread in a thin layer and exposed to the air at the ordinary temperature of the room.

Sifting.—A sieve with round holes 3 millimetres in diameter shall be used to separate the fine earth for analysis from the stones and gravel. Gentle pressure with a wooden or caoutchouc pestle, or other means, may be adopted to break up aggregates of clay and silt, but care should be taken not to crush any of the stones or lumps of chalk.

For determination of the "available constituents" the "fine earth" is used without grinding. For the other determinations 100 grammes or more of "fine earth" is sifted through a woven sieve of 40 meshes to the inch, or a sieve with round holes of 1 millimetre in diameter. What is retained by the sieve is ground till it will pass through, and the whole mixed.

Determination of Carbonate of Lime.—The carbon dioxide evolved on treatment of the fine earth with acid is calculated as carbonate of lime.

Determination of Total Mineral Constituents.—The fine earth is boiled with strong hydrochloric acid in an open flask for a short time in order that the acid may attain constant strength, and then digested at the ordinary water-bath or steam-oven temperature for forty to forty-eight hours, the flask being loosely stoppered. In this solution the phosphoric acid and potash are determined, and other mineral constituents as desired.

The object is to obtain as thorough an extraction of the soil as is possible, short of ultimate analysis. The period for the extraction is made sufficiently long to minimize errors due to variations in the actual time, the strength of the acid, or the temperature.

The Bacteriological Examination of Soil.

From the point of view of an examination to obtain results immediately available for public health purposes, the bacteriological examination of soil has only a limited usefulness.

Its utility from this aspect is chiefly in connection with the contamination of water from surface washings.

The examination of soil for *B. typhosus*, *B. pestis*, and other pathogenic organisms is, apart from the pathogenic anaërobes, a matter of great difficulty. The examination is made on the general lines laid down for the isolation of these organisms. Thus, to detect the typhoid bacillus in soil, the soil is mixed thoroughly with sterile water, and the water examined for this organism by methods similar to those used for its isolation from contaminated water.

A number of investigations have been carried out upon the vitality of typhoid bacilli in soil. The results show considerable discrepancies. They indicate that under experimental conditions the typhoid bacillus will survive for many weeks (ten to twelve) in soil, but that under natural conditions probably not more than one week; and that the factors influencing its vitality are many and varied, the antagonism of other microbes, and the physical conditions of moisture and temperature being the most important.

Soils which have been comparatively recently contaminated with organic matter in quantity—for example, by sewage or manure—show evidence of this when bacteriologically examined in the total number of aërobic organisms, the number of spores present, the number of *B. coli*, *B. enteritidis sporogenes*, and streptococci.

The following statement is by W. G. Savage:

"In collecting soil for bacteriological examination the depth from which it is obtained is of fundamental importance. If the surface soil is to be examined, scrape up with a sterile spatula, and transfer to a sterile receptacle. To obtain soil from a given depth either a fresh cutting must be made and the soil collected at the required depth, or, preferably, some form of borer may be used. For this purpose Fraenkel's borer is convenient, its chief drawback being that it holds only a small quantity of soil.

"If Fraenkel's borer is used, it is advisable to collect at least eight samples from spots about a foot apart, and to mix together

to obtain a representative sample. Also in this way sufficient soil will be obtained for a concurrent chemical examination. By means of this borer the exact depth of the soil taken can be ascertained. Owing to its length it cannot be sterilized in the hot-air oven, but it can be conveniently and sufficiently sterilized by pouring in methylated spirit and igniting. After sterilization wrap the lower portion in a sterile cloth and secure with string. This plan is very convenient when a number of samples have to be taken in one day, and at, perhaps, a long distance from the laboratory, since the borer can be re-sterilized at once before each sample is taken, it being only necessary to carry a

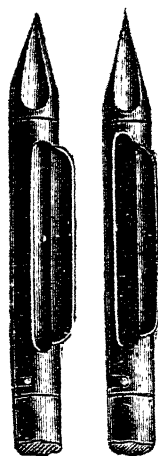



FIG. 20.—FRAENKEL'S BORER.
Lower end shown with open and closed soil-chamber.

bottle of spirit and a number of sterile cloths in a metal box. The soil is removed by a sterile spatula from the interior of the borer to the sterilized tin or other receptacle used for the soil.

"The examination should be commenced as soon after collection as possible.

"To estimate the total number of bacteria, and for some other steps of the examination, very extensive dilution must be practised. As an example of a convenient method of dilution the following procedure is given: other methods of dilution will readily suggest themselves. It is important to remember that owing to a number of inherent difficulties (such as the difference of coherence of different soils) numerical estimations are only

relatively accurate and in any case the same method should be used throughout for each investigation:

"Accurately weigh a small sterile glass-stoppered bottle containing 100 c.c. sterile water. Quickly introduce, with a sterile spatula, 1 gramme of the soil (previously well mixed together) into the bottle. With a little practice 1 gramme can be quickly and sufficiently accurately added. Mix very thoroughly by repeated shaking, if necessary breaking up the soil by a pointed sterile glass rod. Call this solution 'Dilution A.' Allow the soil particles to settle, then add 1 c.c. or more, according to the suspected contamination of the soil, to a sterile flask containing 100 c.c. (or, more accurately, 99 c.c.) sterile water. Mix thoroughly and label 'Dilution B.' Varying quantities of Dilutions A and B are used for the examination.

"To obtain the total number of aërobic organisms make gelatine plates from these dilutions. Thus, 0·2, 0·5, 1·0 c.c. Dilution B are convenient amounts to add to the gelatine tubes. For the number of organisms developing at 37° C. use in the same way agar plates.

"For the number of spores, present as such, add varying amounts of the dilutions to gelatine tubes. Heat to 80° C. for ten minutes, then plate, incubate, and count in the ordinary way.

"For *B. coli* various fractions of the dilutions are added to tubes of lactose bile salt media. These are incubated at 37° C., and those which produce acid and gas are used to inoculate solid media, and the organism isolated exactly in the same way as for the isolation of this bacillus from water.

"Streptococci and spores of *B. enteritidis sporogenes* are examined for by methods identical with those used for water."

According to Houston and Savage, *B. coli* is absent, or present in small numbers only, in uncontaminated soils, and is not readily isolated even from soils polluted with objectionable animal matter, unless the contamination is gross in amount and of *recent* sort. Houston found that when sewage containing large numbers of *B. coli* is added to soil the coli organisms relatively rapidly disappear.

Houston regards the presence of the spores of *B. enteritidis sporogenes* as an indication of contamination, but not necessarily recent, and the presence of streptococci as indicating very recent contamination. Experimenting with soil to which sewage (containing numerous streptococci) had been added, he found that the addition of sewage to a soil might be detected by the presence

of streptococci even in a minimum amount of the soil thus polluted, but that their disappearanceseems to be extremely rapid.

The results, obtained by the writer, of a mineral analysis of a few common soils are given below. It must be understood that soils which are called by the same name may vary considerably in the nature and amounts of their less characteristic constituents. The main purpose of the following analyses is to afford an *approximate* idea of the amounts of the more characteristic substances which enter into the composition of a few of the more common soils:

CLAY (*Stourbridge*).

Silica	68
Alumina	15
Organic matter	4
Iron (oxide)	3
Lime	1·5
(carbonate, 1·4)	
(sulphate, 0·1)	
Magnesia, etc. / Phosphoric acid	traces 0·5
Water	8
	100·0

CALCAREOUS (*Sussex*).

Lime	90
(carbonate, 89·5)	
(sulphate, 0·35)	
(phosphate, 0·15)	
Organic matter	3
Oxide of iron and alumina	2·5
Silica	0·55
Magnesia (carbonate)	0·5
Water	3·45
	100·00

PEATY (*Devonshire*). (Air-Dried Soil.)

Organic matter	90·5
Silica	7·5
Alumina	0·74
Lime	0·5
Oxide of iron	0·46
Sulphuric acid	0·2
Magnesia	0·05
Soda and potash	0·03
Phosphoric acid	0·02
	100·00

Garden (Vegetable) Mould.

Silica	49·25
Organic matter	13·5
Oxide of iron	9·25
Carbonic acid	7·12
Lime	5·13
Alumina	2·74
Soda and potash	2·5
Chlorine	1·5
Sulphuric acid	1·3
Phosphoric acid	0·4
Oxide of manganese	0·25
Magnesia	0·16
Water	6·9
	100·00

PART IV

AIR ANALYSIS

CHAPTER I

THE NORMAL CONSTITUENTS OF AIR—OXYGEN—EUDIOMETRY

COMPOSITION OF THE ATMOSPHERE (freed from aqueous vapour):

	In 100 Volumes.
Nitrogen	78·07
Oxygen	20·95
Argon	0·95
Carbonic acid	0·03
Hydrogen	traces.
Ammonia	traces.
Nitric acid	traces.
Helium, krypton, neon, xenon	traces.

The amount of aqueous vapour is variable, the average in this country being 1·4 per cent.

Suspended matter is also present in variable nature and amount.

In the **air of towns** the carbonic acid may vary from 0·03 per cent. to 0·08 per cent. and over during the prevalence of a fog. Oxidized sulphur and sulphuretted hydrogen are frequently present in traces, as are also ammonia, marsh gas, and organic matter. During dense fog in large town districts the amount of sulphurous and sulphuric acid present is very much increased.

The air of large towns is generally slightly acid, owing to the sulphurous and sulphuric acids which are derived from the sulphur compounds contained in the articles used for combustion; and the air of occupied rooms in which coal gas is burning may be slightly acid. A piece of delicate blue litmus-paper,

moistened with neutral distilled water, commonly denotes this acidity by changing in an hour or less to a faint, though distinct, red.

Oxygen.

We have seen that the amount of oxygen in the external atmosphere may be taken to constitute a normal percentage of about 20·95. After a careful consideration of the number of investigations which have been made, it seems that it may reach a slightly higher limit over large expanses of open country, and that, even in the atmosphere of occupied rooms, it rarely falls below 20·75 per cent.

In some mines the oxygen has been estimated considerably below 20 per cent. (Angus Smith found 18·27 per cent., and some continental observers have estimated it even lower.)

The Estimation of the Amount of Oxygen in the Atmosphere.

Eudiometry.

Apparatus required. — An eudiometer (ευδῖος, good, and μέτρον, measure) is the instrument employed for measuring the volume of a gas or gaseous mixture. One of the most simple forms is Hempel's gas burette, and this will suffice for the purpose of indicating the principles of an eudiometric observation. From the accompanying figure it will be seen to consist of two glass tubes supported on flat stands and connected together at their lowest points by wide india-rubber tubing; the tube which is seen in Fig. 21 to be held up (and which will be subsequently referred to as tube A) is plain, and is continued full bore to the top, where it generally ends in a slightly trumpet-shaped mouth; the other tube (which will be referred to as tube B) is graduated into c.c., and narrowed above so as to fit inside of a short piece of small india-rubber tubing, which serves to connect it with an "absorption pipette" containing the absorbing solution. This apparatus, as shown mounted upon a wooden stand, consists of two glass bulbs blown in a fine glass tube, bent in the manner portrayed in the figure; the lower globe has a larger diameter than the upper, and is capable of holding about 150 c.c. of the reagent employed, while the upper one should be of at least 100 c.c. capacity.

To charge the absorption pipette the liquid reagent is poured into the upper bulb, and the air is then sucked out of the lower bulb through the capillary tube rising from it, until the lower bulb is filled and the reagent reaches into the siphon bend of the capillary tube, but leaves the upper bulb almost empty.

The single "absorption pipette" is shown in Fig. 21; but for the absorption of oxygen it is necessary to use "a double pipette." Reagents that are affected by oxygen (such as pyrogallic acid and cuprous chloride) should not be employed in a single pipette,

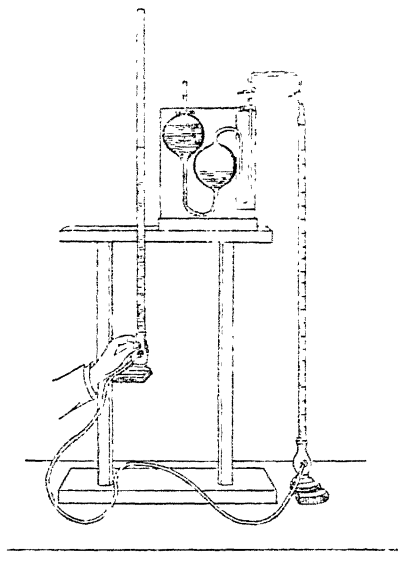



FIG. 21.—HEMPEL'S GAS BURETTE AND ABSORPTION APPARATUS.

because the reagent in the lower bulb is exposed to the general atmosphere. Hempel's double pipette permits of the use of such reagents without this exposure. The double pipette is shown in Fig. 22. The first bulb is the largest (150 c.c.), and is filled with the absorbent; the next is empty; the third contains water; and the fourth is empty. Thus, when any gases are passed over into the "pipette," the water in the third bulb passes into the fourth to make room for the gas, and thus shuts out the atmosphere, the absorbing reagent only coming in contact with the small amount of air originally in the second bulb.

Reagents Employed.—A solution of pyrogallic acid and caustic soda—*i.e.*, 15 grammes of the acid and 50 of caustic soda to the litre of distilled water.

The Process.

1. A certain amount of atmospheric air is first measured in the gas burette in the following manner: Both tubes are placed upon a level surface, and distilled water, which has been thoroughly shaken up in the air and thus mechanically saturated with it, is poured down the plain tube A until each tube is about half-full. Now if the tube A is raised the height of the water in B will ascend until it fills the tube B; and when the tube A is subsequently lowered the atmospheric air of the compartment will pass into the graduated tube B, where it can be imprisoned by turning the greased cock at its mouth. The volume of air thus collected is next exposed to the same atmospheric pressure as obtains in the room by adjusting both tubes so that the water stands at the same level in each. An accurate reading is then taken of the volume of air collected. It is convenient to take about 100 c.c.

2. Connection is then made, as shown in the figure, by fine, stiff india-rubber tubing, between the burette and the "absorption pipette"; the latter being raised close to the top of the tube containing the air, since it is desirable to have as short a length of tubing as possible.

3. A background of white enamel serves to make the coloured absorbing liquid which rises in the capillary tube distinctly visible, and enables the precise height to which it reaches to be carefully marked.

4. Next, by liberating the clasp upon the tubing and opening the greased cock above referred to, the gas burette and absorption pipette are put into communication with each other, when by raising the tube A the air is forced over into the absorption pipette. The cock on the burette is then closed, the india-rubber tubing is pinched, and a firm clasp applied, after which the pipette may be disconnected and gently shaken.

5. When absorption has taken place, by reconnecting the absorption apparatus with the burette, opening the cock and removing the clasp, the residual air can be brought back into the burette by lowering the tube A, care being taken that the

absorbing solution does not pass beyond where it originally stood in the connecting capillary tube, as indicated by the mark on the porcelain.

6. The air may be thus treated several times, in order to give the solution time to absorb all the oxygen.

7. Before making the final reading the height of the water in the two tubes is brought to the same level, just as it was at the commencement of the process (and for the same reason), and then the volume which the air *now* occupies is read off. A constant reading should be obtained.

8. The volume remaining is due to nitrogen, and the difference between it and the original volume represents the oxygen and CO_2 absorbed. The solution of pyrogallic and caustic potash will also absorb sulphuretted hydrogen, sulphurous acid,

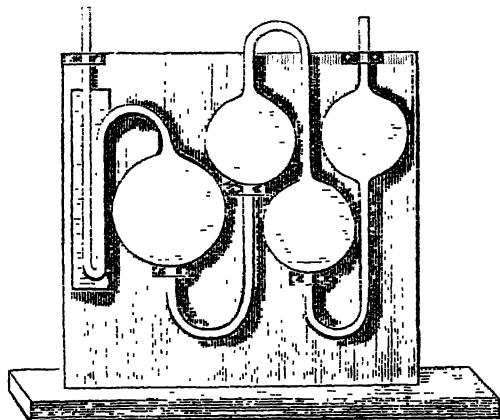



FIG. 22.—HEMPEL'S DOUBLE ABSORPTION PIPETTE.

and hydrochloric acid (if present). From the percentage loss in the volume of the air by this treatment the percentage amount of CO_2 (estimated by Pettenkofer's process, and calculated to the same temperature and pressure) must be deducted, and the remainder will represent the percentage amount of oxygen at the current temperature and pressure.

Example.—The volume of air collected in the burette at the current temperature and pressure was 50·6 c.c. The volume of the residual air after treatment was 40·0 c.c. Therefore the loss is 10·6 c.c. in 50·6 c.c.=approximately 20·59 per cent. Assuming that the CO_2 in the air of the room has been found to be 0·05 per

cent., the oxygen would amount to approximately (20·95 − 0·05) = 20·90 per cent., at the current temperature and pressure.

Notes.—At temperatures of about 15° C. the last trace of oxygen is thus removed in about three minutes of shaking (Hempel).

Since the conditions of temperature must remain the same throughout the estimation, the gas burette, after it has been charged, should not be handled except by its iron or wooden stand, and the apparatus must not be moved about from one spot to another.

The absorbing reagent should always first be saturated at the current temperature and pressure by shaking it up with gases that are but slightly soluble in it, otherwise errors of estimation result; the necessity of always thus saturating the water in the burette with the gas under examination has been mentioned. With this precaution this method of eudiometry gives results but little inferior to those obtained by working over mercury and using solid absorbents.

Mr. J. F. Spencer has designed a four-way tap and connections, fitted to the top of the burette, which permits of the absorbing liquid being brought right up to this tap, so that on turning the tap the gas in the burette can be brought directly in contact with the absorbing liquid, without any intervening air space.

The oxygen may also be estimated by **Dumas' process,** in which the air, having been freed of its carbonic acid by aspirating through a strong solution of caustic potash, is passed through a combustion tube containing a length of pure spongy metallic copper. The copper is kept ignited, and becomes tarnished by oxidation; the difference in weight of the original copper and the tarnished cold metal represents the oxygen taken up from the volume of air experimented upon.

CHAPTER II

CARBONIC ACID

THE estimation of the carbonic acid in the atmosphere is of great value. This is not because the carbonic acid is liable to exist in injurious amounts even under the worst conditions of ventilation commonly obtaining, but because this gas, when furnished by respiration, may afford an important clue to the general condition of the atmosphere.

It is therefore the knowledge of *the amount of carbonic acid which has been added to the atmosphere by respiration* which is generally required.

So inert is carbonic acid *per se* that it may exist to the extent of 2 to 3 parts per 100 without serious consequences, and fatal results would not accrue with less than from 5 to 10 per cent.

The amount of carbonic acid which is present in a pure atmosphere, and which may be termed "normal," is 0·033 per cent. by volume.

The lowest estimation of carbonic acid made in any atmosphere was 0·02 per cent., in air at a very high altitude.

The external atmosphere of cities, during fogs, often contains 0·07 per cent., and may contain as much as 0·09 per cent. or even more.

In ill-ventilated sitting-rooms, well lighted by gas, the carbonic acid often reaches 0·2 per cent.

Where there is "overcrowding" it has been estimated as high as 0·7 per cent., and it is commonly under these circumstances 0·3.

Angus Smith found in the worst parts of theatres 0·32 per cent.

We are indebted to Pettenkofer for a method of estimation which, owing to the facility of its performance and the accuracy of its results, is very generally adopted.

The Collection of Samples for the Estimation of Carbonic Acid by Pettenkofer's Method.

For the estimation of carbonic acid, the samples of air may be conveniently collected in wide-mouthed, glass-stoppered bottles of about 4 litres capacity, which, when used for this purpose, are termed "air-jars." These must be thoroughly cleansed in every case before use, and the last washings should be with ammonia-free distilled water. After the collection of the sample the stoppers should be tied down, and hermetically sealed with prepared lard in those cases where they have to be removed. Lastly, a label is attached, on which should be written a statement of the current temperature and pressure, the date, the hour, the place from which the sample was taken, and the nature and extent of the non-human sources of CO_2 (gas-burners, candles, and lamps).

Following out the principles advocated with regard to water samples, a sample of air should be collected—whether it be vitiated by respiration, combustion, trade processes, or by products of decomposition, etc.—at the time when, so far as can be judged, the atmosphere will afford its maximum evidence of pollution. In investigating the respiratory contamination of the air of a bedroom, for instance, the sample should be taken shortly before the first riser quits the room—that is to say, after the room has been occupied by its customary number of occupants for the greatest number of consecutive hours.

The immediate proximity of gas, lamp, and candle lights, stoves and fireplaces should be avoided; and samples should always be taken at the mean height at which the air is being respired.

For purposes of calculating the *added* CO_2 a comparison sample of the external atmosphere should always be taken at about the same time, since the CO_2 in the external atmosphere in towns varies materially from time to time.

The air is made to occupy the jar by either of the following methods:

1. An air-jar may be accurately filled with clean water—which can, with rare exceptions, be got upon the premises, and should have been previously boiled—and then emptied and allowed to drain in the compartment the air of which is to be examined. A sample of the air then rushes in to fill the place of the escaping

water. At the time of use the water should be at the temperature of the room.

2. The air may be forced in by bellows, which are provided with a long nozzle, which reaches well down into the jar to within an inch of the bottom. This insures that the air which originally occupied the jar will be completely displaced from below upwards.

3. The original air in the jar may be pumped out by means of a small air-pump.

Angus Smith drew the air out of the bottle by a flexible bellows-pump, shown in Fig. 23.

For filling small bottles, J. S. Haldane suggests a long piece of rubber tubing reaching from the bottom of the air-jar to the operator, who sucks in a deep breath of air through the tube.

FIG. 23.—THE FLEXIBLE BELLOWS-PUMP EMPLOYED BY ANGUS SMITH TO DRAW OUT AIR FROM THE AIR-JAR.

4. A jar may be accurately filled with mercury, and emptied in the compartment where the sample is to be collected. Although this plan is theoretically the best, it is practically inapplicable on account of the large amount of mercury required, and the difficulty of conveying this (from its great weight) from place to place.

The first method is recommended; for it is easy of execution, and furnishes satisfactory results.

Whenever it is possible, there is a slight advantage in making the analysis at once in the compartment in which the sample has been taken, since the general atmosphere is that of the jar; and this can sometimes be done, although it is often very

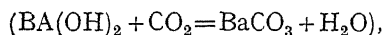
inconvenient. There should be as little loss of time as possible in commencing the analysis, and in the meantime the jar should not be exposed to temperatures varying much from that at which the sample was collected.

The room in which the sample is analyzed must be free from draughts and of a uniform temperature, or, at least, not liable to frequent changes in temperature.

The error which would be introduced by breathing into the air-jar and reagents, or by handling the jars more than is absolutely necessary with warm hands, is obvious. It is desirable to hold the breath for the few moments during which the sample of air is being collected in the air-jar, the wide mouth of which permits of a rapid escape of its contained water.

PETTENKOFER'S ALKALIMETRIC METHOD OF ESTIMATING THE CARBONIC ACID IN THE ATMOSPHERE.

The rationale of the process is as follows: Clear baryta water combines with carbonic acid with great readiness, thereby becoming turbid

$$(BA(OH)_2 + CO_2 = BaCO_3 + H_2O),$$

and the carbonic acid taken up reduces the alkalinity of the baryta water. If, therefore, the degree of alkalinity of a measured quantity of baryta water is estimated, and then this reagent is made to take up all the carbonic acid of a sample of air, the amount of carbonic acid taken up will be in proportion to the reduction of alkalinity of the baryta water.

Special Apparatus:

1. An air-jar of about 4 litres (4,000 c.c.) capacity. It is necessary to know the exact capacity of the bottle in order that the amount of air which it will hold may be accurately known. This can be ascertained by filling the bottle with as much water as it will hold when the stopper is inserted, and then measuring the water as it is emptied out; the volume of the water which the bottle held will correspond to the volume of air which takes its place.

Chemical Reagents:

1. Pure clear baryta water (4·5 grammes of the crystallized hydrate to the litre) to which about ½ gramme of baric chloride should be added to counteract the influence of small quantities of alkalies which may be present.

In order that the baryta water may be kept quite pure it will be necessary to remove the carbonic acid from the air which enters the store bottle when some of its contents are withdrawn, by making it pass through soda

lime. Fig. 24 shows how this can be readily effected: A large glass store bottle is represented, fitted with a siphon tube to draw off the clear baryta water. Any air which enters must pass through the tube, which is packed with soda lime.

2. A standard solution of oxalic acid (crystallized) made to such a strength (*i.e.*, 2·819 grammes to the litre) that 1 c.c. corresponds to 0·5 c.c. of carbonic acid at the standard temperature and pressure.

3. A solution of phenolphthalein (1 part in 250 parts of alcohol).

The Process.

1. A sample of the air is collected in the air-jar.

2. Fifty c.c. of perfectly clear baryta water are then placed in the jar, and the liquid is made to flow round the sides of the jar

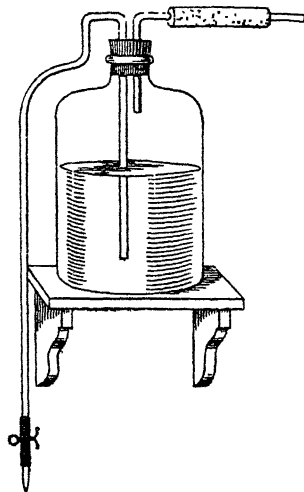



FIG. 24.—STORE BOTTLE FOR BARYTA WATER.

by rolling it about on its side for a minute or two from time to time. About three-quarters of an hour should be allowed for *all* the carbonic acid present in the sample to combine with the baryta.

3. The alkalinity of another 50 c.c. of the clear baryta water is meanwhile tested in the following manner: The 50 c.c. are placed in a small flask with a very narrow neck, and are tinted pink by a drop of phenolphthalein; then the standard solution of oxalic acid is cautiously run in from a graduated burette until the alkalinity of the baryta water has just been neutralized by the

acid, when the amount of acid employed is read off from the burette. The exact neutral stage is indicated by the disappearance of the pink colour.

4. At the end of three-quarters of an hour the causticity of the baryta water in the air-jar is estimated as above. The precipitate of barium carbonate present does not materially affect results with the small amounts of CO_2 generally estimated.

5. The difference in the number of cubic centimetres of acid solution required to neutralize (*a*) 50 c.c. of the original baryta water, and (*b*) the 50 c.c. of baryta water which has taken up the carbonic acid of the air in the jar, represents the amount of carbonic acid in the air.

6. When 50 c.c. of baryta water were added to the jar an equivalent bulk of air was displaced. The amount of air experimented upon, therefore (assuming the capacity of the jar to be 4,000 c.c.), represents 4,000 − 50 c.c. = 3,950 c.c.

7. The result must be returned as the amount of carbonic acid per cent. of the air at the "standard temperature"—*i.e.*, 0° C.—and the "standard pressure"—*i.e.*, 760 millimetres of mercury—since the value of the oxalic acid solution is in respect to CO_2 at the standard temperature and pressure.

Example.—The causticity of 50 c.c. of the original and clear baryta was tested by cautiously running in the standard acid solution. Twenty c.c. of the acid solution were required to effect the neutralization.

The 50 c.c. of baryta water in the air-jar required 17.

∴ 20 − 17 = 3 c.c. of the acid solution represents the carbonic acid taken up by the baryta water from the sample of air.

But 1 c.c. = 0·5 c.c. of carbonic acid.

∴ 3 c.c. = 1·5 c.c. of carbonic acid.

∴ the carbonic acid originally in the air sample amounts to 1·5 c.c. at the standard temperature and pressure.

The capacity of the jar was 4,000 c.c., and the air examined is 4,000 c.c. − 50 c.c. = 3,950 c.c. at the current temperature and pressure—say, 742 millimetres and 17° C. Now, this volume of air has to be converted to its volume at the standard temperature and pressure. By Boyle's law the volume of air varies inversely as the pressure. By Charles' law the air contracts on cooling $\frac{1}{273}$ (= 0·00366) *of its bulk at* 0° *C.* for every degree Centigrade down to 0°.

The volume of air experimented with will therefore represent, at the standard temperature and pressure:

$$\frac{3950 \times 742}{760 \times \{1 + (0 \cdot 00366 \times 17)\}} = \frac{2930900}{760 \times 1 \cdot 06222} = 3{,}630 \text{ c.c.}$$

or, $3950 \times \frac{742}{760} \times \frac{273}{290} = 3{,}630$ c.c.

∴ there were 1·5 c.c. CO_2 in 3,630 c.c. of air, or 0·0413 per cent., at the standard temperature and pressure.

The sample of the outside air is similarly examined, and the difference between the CO_2 in the inside and outside air will represent the added CO_2 impurity of the inside air.

Notes.—One c.c. of CO_2 at 0° C. weighs 1·96633 milligrammes at 760 millimetres pressure; the relation, therefore, between the volume and the weight$=\frac{1}{1 \cdot 96633}=0 \cdot 508$.

The writer prefers to perform this process in a long cylindrical jar of the capacity of about 3 litres and fitted with a doubly perforated cork, one perforation transmitting the drawn-out point of a graduated burette with stopcock, and the other perforation a small piece of glass tubing carrying externally a small, close-fitting, india-rubber cap. The air is collected as directed; 50 c.c. of baryta water (standardized at the time of use by the standard oxalic acid) is added through the glass tube, which is then sealed by its cap. After one hour, the bottle being gently rolled along a table from time to time in the meanwhile, a few drops of phenolphthalein are added through the small glass tube (the cap of which is now removed), the burette is charged with the standard oxalic acid solution, and this is allowed to discharge cautiously into the bottle. So soon as the pink colour commences to weaken, the acid is added slowly in drops, and the bottle is gently shaken and stood upon a white porcelain slab. The neutral stage can be readily noted when the experimenter looks down from above on to the white porcelain slab. This method avoids exposing the baryta to the atmosphere and the breath of the worker.

The above method, in which the air is collected in a large bottle, is preferable to the method of slow aspiration of air through barium hydroxide contained in tubes, for it is more convenient in practice, and the sample represents the state of the air at a particular time; whereas by the latter method the air is being continuously collected for an hour or more.

Letts and Blake have pointed out that the action of the BaH_2O_2 on the glass leads to a slight contamination with alkalies and silica, and therefore some error of experiment. They recommend that the air-jar and the baryta stock bottle should therefore be coated with paraffin wax; but the writer finds that this precaution may be disregarded in the case of bottles which have been previously well exposed to BaH_2O_2.

Haldane's Rapid Method of Determining Carbonic Acid in Air.

In this process the apparatus shown in Fig. 25 is required. The gas burette A, which is enclosed in a water-chamber, consists of a wide ungraduated and a very narrow graduated portion. It holds about 20 c.c. from the tap to the bottom of the scale. The graduated part, which is about 4 inches long, is divided into 100 divisions, each of which corresponds to $\frac{1}{10000}$th part of the capacity of the burette. The lowest division is marked 0, and the highest 100. Any difference between a reading at or near zero and a second reading is thus shown by the scale in volumes per 10,000.

In using the apparatus the air is first expelled from the gas burette by opening the three-way tap B to the outside and raising the mercury bulb C. The tap is then closed, and the mercury bulb replaced in its stand. On opening the three-way tap again a sample of the air is drawn in, and the level of the mercury falls to near the zero mark. The tap is now opened towards the absorption pipette D, which is filled up to a mark at E with a 20 per cent. potash solution, and the sample measured with the precautions to be described below. It is then passed over (by raising C) into the absorption pipette, the potash being displaced into bulb I. The air is driven backwards and forwards for a minute, and then again measured after the absorption of the carbonic acid. The difference between the two readings gives directly the number of volumes of carbonic acid per 10,000 in the sample of air.

It is evident that the correctness of the analysis depends entirely on the avoidance of errors of various kinds in the two determinations of the volume of the enclosed air. Mistakes might be caused by slight variations in the temperature of the water, or the pressure under which the sample is measured, or

in the degree of saturation with moisture of the sample. A variation of 0·1° C. in the temperature of the water in the jacket would, for instance, unless corrected, cause an error of fully 4 volumes per 10,000 in the analysis.

In order to have a sharp index of the pressure under which the air is measured, the level, not of the mercury, but of the potash solution in the narrow bore tubing of the absorption pipette, is taken as the index of pressure. For the first measure-

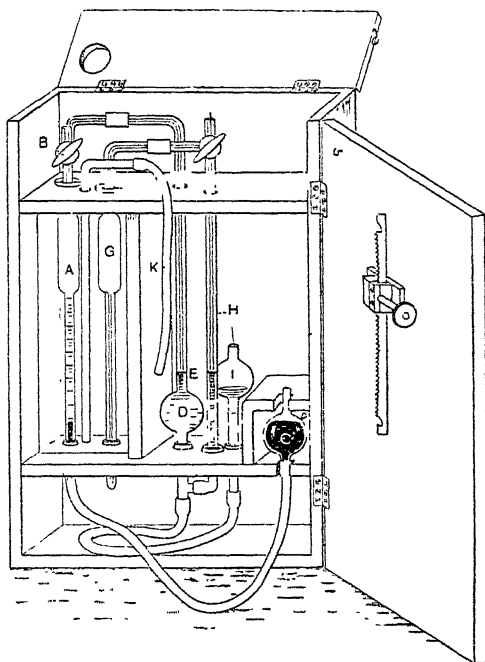



FIG. 25.—HALDANE'S APPARATUS.

ment, the level of the potash solution is accurately adjusted to the mark at E by raising or lowering the mercury by means of the rack-and-pinion arrangement shown in Fig. 25. For the second reading the potash level is again adjusted in the same way.

To correct for variations in temperature of the water-jacket a control tube G is employed, of a size and shape approximately the same as the gas burette. The control tube communicates with the potash through the narrow-bore glass tube H, and before the first measurement is made the level of the potash in

H is adjusted to the mark by lowering or raising the reservoir I, which slides up and down in a loosely-fitting cork. At the second measurement the same precaution is taken, so that the air in the control tube occupies exactly the same volume as at the first measurement. As an alteration of temperature or of barometric pressure would affect the pressure to an equal extent in the gas burette and control tube, it is evident that the adjustment of the level of the potash reservoir compensates exactly any error which the alteration of temperature or of barometric pressure would cause in the reading of the gas burette.

Before the adjustments of the potash levels are made, the water in the jacket is thoroughly mixed by blowing air through it by means of the tube K. This manipulation is essential to an accurate result.

In order to obviate error due to variations in the saturation of the air, both the burette and the control tube are left with a little visible moisture inside. If the burette has once been wetted inside, and as much as possible of the water expelled by raising the mercury, it remains moist for a very large number of analyses, but a little moisture should always be visible. If by any mishap potash should be sucked over into the burette, it and its connection must be washed out with dilute acid introduced by the tap.

At the end of an analysis the taps must be turned so as to close the communication between the potash and the burette and control tube; otherwise potash may be sucked in if there is any great fall of temperature or rise of barometric pressure.

The manipulations required during an analysis may be recapitulated as follows: (1) Open the tap of the control tube to the air for a moment, and then turn it so as to connect the control tube and potash-pressure gauge. (2) Turn the tap of the burette so as to connect the burette and the potash pipette. (3) See that the level of the potash alters sharply and about equally in the tubes when the potash reservoir is raised. (4) Blow air through the water-jacket. (5) Raise or lower the potash reservoir till the potash is exactly at the mark in tube H. (6) Raise or lower the mercury reservoir by means of the rack and pinion till the potash in E is exactly at the mark. (7) Read off the mercury level on the scale of the burette to 0·2 of a division. (8) Raise the mercury to the upper hook, so as to drive the air

into the potash bulb, then lower it a little and raise it again twice so as to wash any carbonic acid in the connecting tubing into the potash bulb. (9) Return the air to the burette. (10) Blow air through the water-jacket. (11) Adjust the two potash levels as before, and read off the mercury level. The first reading subtracted from the second gives the result in volumes per 10,000. (12) Close the two taps.

The advantages of the method are that an estimation can be made in two or three minutes, only a very small volume of air is required, and the apparatus, being fitted up in a small box, is exceedingly portable; the disadvantages are that it is relatively costly, it only makes an approximate estimation of the amount of CO_2, and (to a beginner) it is rather difficult in manipulation. The process is, however, sufficiently accurate to suffice for most of the practical purposes of hygiene.

Conclusions to be drawn from the Amount Estimated.—From the writer's experiments, no "stuffy" odour is appreciable in the atmosphere until the respiratory impurity reaches at least 0·03 per cent. in those cases where samples have been collected from rooms occupied under ordinary conditions.

This stuffiness is mainly due to exhalations from the skin, and the degree of personal cleanliness largely determines the rapidity of its appearance; and recent experiments have demonstrated that the *physical* changes in impure air are at least mainly responsible for the ill-effects of the air of overcrowded rooms.

These experiments included a number of tests made in a specially constructed glass chamber in which the physical and chemical qualities of the air could be rigorously controlled. It was found that with a respiratory impurity of carbonic acid exceeding any recorded up to that time as having been found in the air of a crowded room—*e.g.*, from 1·0 to 1·5, or even 1·7 per cent.—no injurious property of the air could be demonstrated so long as the temperature and humidity were kept low; and that under these circumstances the absence of any disturbance was so complete that even the power of cerebration remained intact.

On the other hand, as soon as the temperature and humidity were increased to beyond a certain point, there appeared, both in normal and in diseased persons who were submitted to experiment, the usual symptoms that occur when people are crowded together in one room—*i.e.*, feelings of discomfort, oppression,

lassitude, giddiness, nausea, etc. These symptoms, however, could be relieved at once by reducing the temperature and humidity of the air to normal.

The extent to which gas burned in a common gas-burner may furnish carbonic acid to the atmosphere is between 2 and 3 cubic feet per hour (for 1 cubic foot of gas produces from 0·5 to 0·6 cubic foot of CO_2); and the amount of sulphur compounds thus yielded in a gas well purified is not important hygienically.

The air over burial-grounds, especially when these are crowded, has been said to contain an abnormally high amount of carbonic acid, but the writer's experiments have failed to confirm this.

CHAPTER III

THE ORGANIC MATTER IN THE AIR

THE organic matter in the air includes that given off from the lungs and skin; its composition is very imperfectly understood, but it consists partly of volatile fatty acids and their ethers, and partly of vaporous and suspended matters (epithelial and fatty débris). It is certainly largely oxidizable and nitrogenous, since it will reduce solutions of the permanganate of potassium, and will yield ammonia. It quickly putrefies; and when air containing it is aspirated through sulphuric acid, the organic particles are charred and darken the solution. When collected in large amounts in water it can be precipitated by silver nitrate. Probably the major part is molecular and suspended, since it does not diffuse equally about a room, and tends to fall and settle; and there is no doubt that it is mostly in combination with watery vapour, for substances absorb it according to their hygroscopic powers—*i.e.*, it is absorbed chiefly by wool, feathers, etc., and least by horsehair. It gives a fœtid, " stuffy " odour to the atmosphere, and from the persistence of this odour it is doubtless burnt off but slowly by the atmospheric oxygen; and in small quantities it gives odour to water.

The *processes which we may employ for the estimation of this matter* in air are preferably those which serve to detect the same matter in water. A large measured volume of the air is made to slowly pass through distilled ammonia-free water, which will retain all the soluble and suspended organic material. The water is then tested by Wanklyn's method as to its nitrogenous organic matter, and by Tidy's process as to its oxidizable organic matter—it being borne in mind in the latter test that either nitrous acid, sulphurous acid, sulphuretted hydrogen, or tarry matters, will, if present, also reduce permanganate of potassium.

A convenient *method of performance* (Fig. 26) is to take a small

wash-bottle, partially fill with 250 c.c. of distilled ammonia-free water, and then tightly fit with a doubly perforated india-rubber stopper. Into one perforation a glass tube bent at right angles, with one trumpet-shaped extremity, is accurately fitted, while the other end is made to dip well down into the distilled water; the second perforation conducts another bent glass tube, the end contained within the flask being above the surface of the water, and the other connected directly by india-rubber tubing to a second wash-bottle similarly fitted, and containing another 250 c.c. of the distilled water. This second bottle is connected

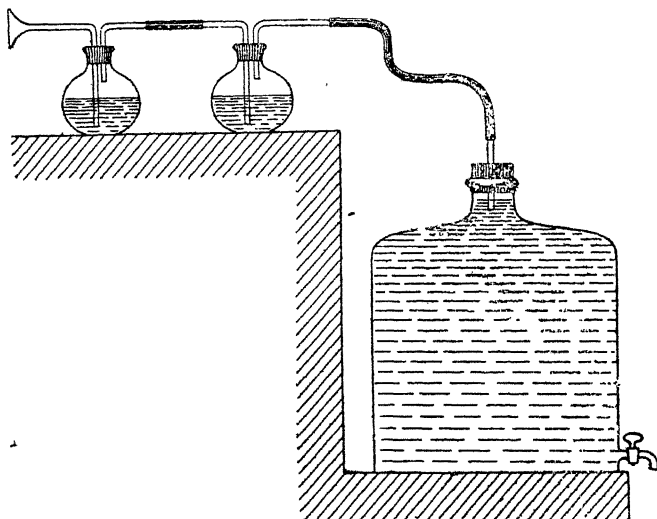



FIG. 26.—APPARATUS FOR COLLECTING THE ORGANIC MATTER IN AIR.

by india-rubber tubing to the aspirator. The capacity of the aspirator being known (and a convenient size is that of 20 litres), it is filled with tap water; the tap is then turned so that the water passes slowly out, when air enters the trumpet-shaped mouth of the bent glass tube to take the place of the escaping water; such air is washed in the distilled water in the two bottles before it reaches the aspirator, and so parts with its organic matter.

Example.—If it is desired to make an estimation of the nitrogenous organic matter, the aspirator is five times filled and allowed to empty; 100 litres of air will then have been drawn through the 500 c.c. of distilled water; therefore this 500 c.c. of water will contain the nitrogenous organic matter of 100 litres of air.

Suppose, after distilling off the free ammonia, the 500 c.c. of water are found by Wanklyn's method to contain 0·02 milligramme of albuminoid ammonia, then there will be 0·02 milligramme of such ammonia in 100 litres of air. But in dealing with air such results are generally expressed in terms of "milligrammes per cubic metre."

Therefore, if there is 0·02 milligramme of albuminoid ammonia in 100 litres, there will be 0·2 milligramme of albuminoid ammonia in 1,000 litres—or a cubic metre—of air.

Outside air contains *albuminoid ammonia* up to 0·1 milligramme per cubic metre, and averages about 0·08. In a hospital ward this ammonia has been estimated as high as 1·3.

The *oxidizable* organic matter may also be collected in the same way, and estimated by Tidy's process, as in Water Analysis.

The estimation of the oxidizable organic matter can also be performed by very slowly aspirating 100 litres of air through two wash-bottles containing standard potassium permanganate solution and dilute sulphuric acid kept at about 27° C. The strength of the permanganate may be millinormal, as in the process next to be described. In fine, bright weather the oxidizable organic matter in town air will absorb about 3 c.c. of oxygen per cubic metre or 3 volumes of oxygen in 1,000,000 volumes of air, but in stagnant and foggy air the amount is considerably higher.

Another method of approximately estimating the organic matter in air is that of Carnelly and Mackie. In this process from 3 to 4 litres of air are shaken with 50 c.c. of millinormal solution of potassium permanganate for five minutes, and the amount of decomposed permanganate is deduced, on colorimetric principles, from the loss in colour sustained by the original solution; and from the extent of this loss, as estimated by the amount of standard solution required to restore the colour, the amount of oxygen absorbed can be calculated. One c.c. of the millinormal solution=0·008 milligramme O=0·0056 c.c., at standard temperature and pressure. To each litre of the $\frac{N}{1000}$ solution are added 50 c.c. of dilute sulphuric acid (1 in 6).

Henriot and Bouissy have suggested that the moisture of a measured volume of the vitiated air of crowded apartments should be collected by condensation, and the reducing substances estimated in the moisture obtained.

The amount of organic matter in air is found to be closely related to the amount of dust.

CHAPTER IV

AMMONIA—MARSH GAS—CARBON MONOXIDE—SULPHUR COMPOUNDS—NITRIC, NITROUS AND HYDROCHLORIC ACIDS—PHOSPHURETTED AND ARSENIURETTED HYDROGEN

AMMONIA.

TRACES of ammonia are present in every atmosphere. In towns it generally amounts to about 0·06 milligramme per cubic metre. Such traces are derived almost entirely from the combustion of coal and coal gas. The ammonia generally exists in combination with an acid, as the carbonate and chloride, or less commonly as the nitrate or sulphate. Ammonia may be found in considerable quantity near ground where decomposing organic matter is deposited. Traces of ammonia do not appear to affect health, but it is a constant ingredient of the most impure airs.

A considerable amount of ammonia in the atmosphere may be detected by moistening strips of filtering paper with Nessler's reagent, and hanging these up for some time in the air of the compartment. But when present only in faint traces, large quantities of air must be aspirated through distilled ammonia-free water rendered slightly acid with sulphuric acid, and the ammonia tested for by Nessler's reagent; and if a measured quantity of air is employed, the ammonia may be estimated quantitatively by "Nesslerization." It has been found as high as 0·8 milligramme per cubic metre in certain hospital wards.

MARSH GAS (CH_4).

This gas probably exists in air in many circumstances, but owing to the difficulties of its detection, it is not easy to prove its presence when in traces only. There are certainly traces in the atmosphere of towns, and over districts of abundant vegetation (especially when such districts are marshy) it may exist in considerable quantities. As evolved from strata in which coal-mining

operations are progressing it is known as "fire-damp," and its power of exploding when ignited in the presence of carbonic acid has been often disastrously exemplified.

There is little doubt that after a while marsh gas may create symptoms of poisoning, and, being inodorous and non-irritating, its presence would not be detected by the senses. Any escape of coal gas, containing as it does some 35 per cent. of marsh gas, will charge the atmosphere with considerable and dangerous amounts, but fortunately in these cases the strongly-smelling ingredients of the coal gas give timely warning.

CARBON MONOXIDE (CO).

The affinity of this gas for hæmoglobin is about three hundred times greater than that of oxygen. Owing to its properties of entering into combination with the hæmoglobin of the red corpuscles, displacing their oxygen and thus paralyzing their oxygen-carrying functions, it destroys life by cutting off the oxygen supply to the brain and tissues; and its dangers are enhanced from the circumstance that it gives no indication of its presence by the sense of odour. Symptoms of poisoning are evident when the hæmoglobin is about one-third saturated with CO, and death results from some 70 or 80 per cent. of saturation.

Sir T. Oliver thus describes the symptoms: In acute intoxication the individual feels dizzy, and complains of headache, noises in the ears, throbbing in the temples, a feeling of sleepiness, and a sense of fatigue. There may be a feeling of sickness which culminates in vomiting, a sense of oppression at the chest, with quickened or irregular breathing, palpitation, and an inability to stand or walk straight. Convulsions may or may not come on, or there may be only a few muscular tremors. There is a peculiar fixed look about the eyes, the pupils of which are dilated and their reaction slow. Consciousness is lost by degrees, or it may be retained for some time, and yet, owing to the great loss of motor power, the individual, although aware of the danger, is often unable to escape from it. When a man has recovered from the effects of carbon monoxide, his life is still imperilled for some days to come. Not only does he run the risk of dying as late as eight days after the accident, but he has still to face the risk of secondary maladies developing—such, for example, as glycosuria.

It may be necessary to examine the air for this gas in the

atmosphere of compartments where iron or copper stoves are employed, and especially when the material is cast-iron and when the fuel is coke; where coal gas (which contains some 5·5 to 6·5 per cent., but may contain from 4 to 12 per cent.) is incompletely burnt or escapes; or where there is a possibility of some of the products of combustion from leaky furnace-flues, etc., escaping into a compartment—for the air in furnace-flues has been found to contain over 20 per cent. of carbonic oxide, and that of ordinary flues from domestic fireplaces from 1·5 to 4 per cent. The carbonic oxide of the air of flues is always the product of imperfect combustion—that is to say, the carbon is either not fully oxidized to carbonic acid (CO_2) owing to the supply of fresh air being insufficient, or else the carbonic acid, being formed low down in the furnace, gets reduced to carbonic oxide in subsequently passing through the rest of the furnace.

Of the gases generated from the explosion of gunpowder, carbonic oxide forms about 7·5 per cent., but in mines it is usually only formed in dangerous amount by an extensive fire-damp explosion, and especially by an explosion in which coal-dust is involved.

A serious drawback to the adoption of "water gas" as a source of heat and light is the fact that it contains (before combustion) from 25 to 35 per cent. of this very dangerous ingredient.

Considerable quantities of CO may exist in the atmosphere near coke-ovens, brick-kilns, and cement works.

The carbonic oxide in the atmosphere of stove-heated rooms is derived from either or several of the following sources:

1. Red-hot cast-iron may transmit the gas from the fire, either through its substance or through minute fissures, and the hot iron may even reduce CO_2 to CO.

2. The carbon which enters into the formation of the cast-iron may get oxidized, and reach the external atmosphere as CO.

3. Particles of suspended organic matter in the atmosphere may get charred and partially oxidized by coming in contact with a highly heated stove.

4. Currents may pass down the smoke flue under certain conditions, and thus introduce the gas.

In the *Lancet* of February 7, 1914, a useful means of testing as to whether the products of combustion of a gas stove are or are not entering a room is described. When traces of chloroform or carbon tetrachloride are introduced into the mixture of air

and gas drawn into a Bunsen burner, the products of combustion then contain hydrochloric acid, which, although present in small proportion, is readily detected by the dense white fumes of ammonium chloride which it forms in coming into contact with ammonia. All that is necessary, therefore, in testing a gas fire as to its ventilating function is to place near the air inlet of the gas fire an absorbent substance (a chalk pencil) containing carbon tetrachloride, and then to hold on a wire a piece of sponge containing strong ammonia solution at different points along the rim of the canopy of the gas fire. Any leakage or escape of combustion products is thus instantly indicated by the production of visible fumes of ammonium chloride. The advantage of this test is that any point of leakage can be located from a gas fire fixed in position in the house.

CO is present in traces in tobacco smoke.

Qualitative Tests.

Vogel's test is sufficiently delicate for all practical purposes.

Into a wash-bottle 100 c.c. of distilled water are poured, and then a little defibrinated blood is added to the bottle, which is afterwards connected to an aspirator. At least 10 litres of air are then drawn through the *faintly* reddish liquid. The test is obscured if too much blood is present. The bottle is then rolled about for half an hour and allowed to stand for a short while,

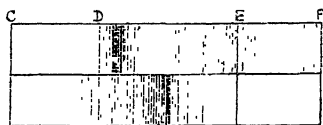



FIG. 27.—SHOWING THE CHARACTERISTIC DISPOSITION OF THE ABSORPTION BANDS IN THE SPECTROSCOPIC PICTURE OF OXY- AND REDUCED HÆMOGLOBIN.

The upper scale represents oxy-hæmoglobin and the lower reduced hæmoglobin.

when some of the contents are removed and examined by the spectroscope.

Oxy-hæmoglobin shows two well-marked bands with sharp edges, in the yellow and in the green parts, respectively, of the solar spectrum, both lying between Fraunhofer's lines D and E. The spectroscopic appearance of hæmoglobin in combination with carbonic oxide is almost identical, but the left-hand band

(at blue end) of the carbonic oxide hæmoglobin lies a little nearer to the right (yellow end) than in the case of oxy-hæmoglobin, and the edges of the band are not so sharply defined. The blood takes up a more or less marked bright pink or cherry-red tint, which may also be observed in the cadaver.

Two drops of a colourless solution of ammonium sulphide are next added, the bottle is well shaken, and the liquid is gently warmed and re-examined. If no marked change in the spectroscopic appearance of the fluid has ensued, carbonic oxide hæmoglobin is *present*; otherwise the ammonium sulphide will deoxidize or reduce the oxy-hæmoglobin, and the two bands will be replaced by a single broad band shaded off at the borders, and occupying a position almost intermediate with regard to the original two bands.

Delicate results are obtained, where the CO is not too small in amount, by placing a mouse in a wire cage and allowing it to breathe the air for several hours. The mouse may be subsequently drowned in its cage, and the blood then examined by the spectroscope to see if the two absorption bands of CO-hæmoglobin are present. A control test may be made from the blood of a mouse which has not been thus exposed to carbon monoxide.

Welzel has devised a delicate chemical test for CO-hæmoglobin: To 10 c.c. of the solution of blood he adds 15 c.c. of a 20 per cent. solution of potassium ferrocyanide and 2 c.c. of acetic acid (1 volume of glacial acetic acid to 2 volumes of water); the precipitate very soon becomes reddish-brown if CO-hæmoglobin be present, but greyish-brown with oxy-hæmoglobin, the difference slowly disappearing.

Chemical Tests upon the Air.

1. If air is aspirated through a tube filled with a solution of palladium chloride (containing 1 milligramme of palladium and 2 drops of hydrochloric acid), a portion of the palladium is reduced to the metallic state and a dark precipitate results. After an hour's action with frequent shaking, the palladium which has deposited owing to the reducing action of the carbon monoxide may be collected and ignited with the usual precautions; 1 gramme of metallic palladium=0·2624 gramme, or 210 c.c. of CO. The air should first be aspirated through lead

acetate solution, and also through dilute sulphuric acid, to remove any SH_2 or NH_3 which may be present.

2. A known quantity of the suspected air, freed from CO_2 by its passage through potash bulbs, is slowly passed over periodic acid contained in a U-tube kept at a temperature of 90° C. The carbon monoxide, if present, decomposes the periodic acid, setting free iodine ($I_2O_5 + 5CO = I_2 + 5CO_5$). From the amount of liberated iodine the quantity of carbon monoxide is deduced. The method is accurate, and no other element likely to be present in the atmosphere will reduce periodic acid; but the air should first be freed from dust and drawn through washing-bottles containing fuming sulphuric acid, aqueous potassium hydroxide, concentrated sulphuric acid, then through two long tubes containing solid potassium hydroxide, so as to remove any carbon dioxide, unsaturated hydrocarbons, and moisture.

Quantitative Estimation.

The subchloride of copper (made by exposing copper turnings and the oxide of copper to the action of strong hydrochloric acid —S.G. 1·124) has the property of absorbing CO, and advantage may sometimes be taken of this fact to estimate the quantity present by the method of eudiometry, where the amount is considerable and exceeds 0·1 per cent.

It is necessary that the O_2 and CO_2 of the air be first removed by means of sodium pyrogallol mixed with a considerable excess of sodic hydrate, before the residual air is slowly passed over into a double absorption pipette charged with the solution of subchloride of copper.

It is also necessary to use an absorption apparatus with large bulbs, in order that a good quantity of the copper solution may be employed; and time must be allowed, for complete absorption takes place slowly. Two or three treatments should be repeated until a "constant reading" is obtained, to insure that all the gas has been absorbed. If there is a marked amount of carbonic oxide present, the loss in the original volume, taken under the same conditions of temperature and pressure, is appreciable, and represents the amount of CO; or the cuprous chloride solution may be transferred, under suitable precautions, and boiled *in vacuo*, and the expelled gas collected. Quite 98 per

cent. of the carbonic oxide actually present will be obtained by the latter method.

The union of cuprous chloride with carbonic oxide is very feeble, and the solution readily parts with the carbonic oxide to the atmosphere on shaking. The solution will also absorb acetylene and ethylene.

J. Haldane's method of testing for the presence and for estimating the amount of carbon monoxide is as follows:

For the detection of CO he places in a dry and clean bottle about 5 c.c. of a dilute blood solution, and then, after aspirating some of the suspected air through the bottle, stoppers it and shakes for ten minutes. During the shaking the bottle should be protected from light, which has a most powerful dissociating action on CO-hæmoglobin. On pouring out the solution into a test-tube and comparing its tint with that of some of the original solution in another test-tube, the presence of the carbonic oxide is indicated by the pink tint of the former.

To measure accurately the extent to which blood is saturated with CO, Haldane's method depends upon the fact that normal blood, when sufficiently diluted with water, has a yellow colour, whereas blood saturated with carbonic oxide forms a pink solution when similarly diluted. A solution of about 1 of normal blood to 100 of water is made; also a solution of carmine, dissolved with the help of a little ammonia and diluted till its *depth* of tint is about the same as that of the blood solution. Two test-tubes of equal diameter (about ½ inch) are then selected. Five c.c. of the solution of normal blood are measured into one of the test-tubes, and a drop of the suspected blood is placed in the other test-tube and cautiously diluted with water till its *depth* of tint is about equal to that of the normal solution. If carbonic oxide be present in the hæmoglobin, a difference of quality in the tints of the two solutions will now be clearly perceptible. Carmine solution is then added from the burette to the normal blood, and water (if necessary) to the abnormal blood, till the tints are equal in both quality and depth. The carmine is added in about 0·2 c.c. at a time, the points being noted at which there is just too little and just too much carmine, and the mean taken. The solution of abnormal blood is then saturated with coal gas by thoroughly shaking up with coal gas for a few seconds, and the addition of carmine to the other test-tube continued until equality is again established, the amount of carmine used being

noted. The percentage saturation of the abnormal blood with CO can now be easily calculated, since we know how much carmine solution its saturation represented as compared with what complete saturation represented.

The method of calculation is illustrated by the following example: To 5 c.c. of normal blood solution 2·2 c.c. of carmine is required to be added to produce the tint of the blood under examination, and 6·2 c.c. to produce the tint of the same blood fully saturated. In the former case the carmine was in the proportion of 2·2 in 7·2, and in the latter of 6·2 in 11·2. The percentage saturation (x) of the hæmoglobin with carbonic oxide is therefore given by the following proportion sum:

$$\frac{6{\cdot}2}{11{\cdot}2} : \frac{2{\cdot}2}{7{\cdot}2} :: 100 : x ;$$

x therefore=55·2. As the compound of CO with hæmoglobin is to a slight extent dissociated when the blood is diluted with water, the value found is a little too low. The corrections needed are as follows: Add 0·5 if 30 per cent. saturation be found, 1·1 if 50 per cent., 1·6 if 60 per cent., 2·6 if 70 per cent., 4·4 if 80 per cent., 10·0 if 90 per cent. Thus, in the above example, we must add 1·3, so that the true saturation is 56·5 per cent. In comparing the tints the test-tubes should be held up against the light from a window, but bright light should be avoided as much as possible, as it increases the dissociation.

For the detection and determination of small percentages of CO in air the sample of air is collected in a clean and dry bottle of about 4 ounces capacity. The cork of the bottle is removed in the laboratory under a 0·5 per cent. solution of blood, and about 5 c.c. of the air allowed to bubble out, a corresponding volume of the blood solution entering. The cork is then replaced, covered with a cloth to keep off the light, and shaken continuously for about ten minutes, when the hæmoglobin will have reached the point of saturation corresponding to the percentage of CO present. The solution is then poured out into a test-tube, and the saturation determined with carmine solution in the manner described above. It is evident that as in each case the saturation found corresponds to a definite percentage of CO in the air, it is easy to calculate this percentage.

The method furnishes good results with very small percentages of CO, but becomes less and less accurate as the amount exceeds 0·2 per cent.

SULPHUR COMPOUNDS.

Sulphurous and sulphuric acids, sulphuretted hydrogen, and ammonium sulphide may all be present in the atmosphere of large towns, the first two invariably so, in traces; the two latter are, however, less often appreciable.

The external atmosphere of towns obtains sulphur compounds from the combustion of coal and gas.

Their presence may be deleterious to health, and the oxy-acids of sulphur are unfavourable to vegetation and destructive to stone-work and mortar, upon which a scale of soluble calcium sulphate forms, and, favoured by the action of rain, the stone disintegrates.

Angus Smith considered sulphuretted hydrogen "one of the most deadly of gases," and held that, in traces even, "it lowers the tone of health."

Sulphuretted hydrogen, in large quantities, has been ascribed as the direct cause of death among sewer-men by Stevenson, Haldane, and others. It is certain that an atmosphere containing from 0·7 to 0·8 of SH_2 per 1,000 of air is dangerous to human life. The gas acts upon the nervous system, and causes a functional arrest of the respiratory centre in the medulla.

SH_2 may also be found in considerable amount in domestic chimneys where combustion is imperfect.

Sulphurous acid in large quantities appears to favour the development of—even if it does not induce—bronchitis, asthma, anæmia, conjunctivitis, etc. It may be estimated by aspirating a known quantity of the air through a dilute solution of bromine in water, precipitating the sulphuric acid thus formed by barium chloride solution, and calculating the SO_2 from the amount of $BaSO_4$ obtained; or by direct titration with one-thousandth normal iodine solution. From 10 to 17 milligrammes per cubic metre of air have been estimated under the worst atmospheric conditions of London and Manchester. A cubic metre of air weighs 1,293,200 milligrammes, and so this amount represents from 0·008 to 0·013 part per 1,000.

Sulphuric acid may be collected by aspirating a large volume of air through distilled water, and the estimation may be made by precipitating it as $BaSO_4$, as described under Water Analysis.

Sulphuretted hydrogen and ammonium sulphide may sometimes be detected by exposing to the air strips of filtering paper

moistened with a solution of lead acetate. Any faint evidence of darkening about the borders of the previously white paper will prove the presence of these gases in the atmosphere. If the darkening is due to ammonium sulphide, filtering paper moistened with a solution of the nitro-prusside of sodium will show evidence of violet coloration if the gas is present in sufficient quantity.

A quantitative estimation of sulphuretted hydrogen may be made by aspirating a measured volume of air through a little freshly prepared decinormal solution of iodine in iodide of potassium, to which some starch paste has been added. The operation is stopped as soon as the solution becomes colourless ($H_2S + I_2 = 2HI + S$). Each c.c. of the iodine solution employed $\times 1 \cdot 7 =$ milligrammes H_2S in the volume of air examined.

The vapour of CS_2 may be absorbed in a strong solution of potash in 96 per cent. alcohol; the contents of the flask are then acidulated with a little acetic acid. A small amount of calcium carbonate is next added, in order to nearly neutralize. The faintly acid solution is then mixed with an amount of water similar to the potash solution employed, and a little fresh starch solution is added. A solution of iodine in potassic iodide, containing 1·666 milligrammes iodine per litre, is then run in until a faint blue colour appears. Every c.c. of iodine solution required $=1$ milligramme CS_2 in the volume of air examined.

In testing for **hydrochloric, nitric, and nitrous acids,** large volumes of air must be taken. The acids may advantageously be absorbed in 10 per cent. pure soda lye. The amount of nitric acid in the air is very small. It is most marked after thunderstorms and in the air of towns. The above-mentioned acids may be estimated by the methods described in Water Analysis.

Chlorine and **bromine** may be absorbed in pure 10 per cent. colourless solution of potassium iodide ($2Cl + 2KI = 2KCl + 2I$), and the liberated iodine titrated by decinormal sodium thiosulphate with starch (12·69 milligrammes $I = 7 \cdot 99$ $Br = 3 \cdot 54$ Cl).

Traces of **arseniuretted hydrogen** in the atmosphere may be detected by aspirating air through a solution of cuprous chloride in hydrochloric acid, and then causing it to impinge on a paper impregnated with mercuric chloride, the depth of tint of the yellow stain produced serving to indicate the amount of AsH_3 present.

Phosphuretted Hydrogen.—This gas has in recent years been shown to be given off by ferro-silicon (employed in the manufacture of steel) when this material is exposed to the action of water or moist air. There is very little danger from the low-grade ferro-silicon, but the danger is very great in respect to high-grade ferro-silicon containing about 40 to 60 per cent. silicon. The phosphuretted hydrogen is derived from the calcium phosphide (Ca_3P_2) impurity in the ferro-silicon, which in contact with water or moist air is decomposed with the evolution of PH_3 (phosphuretted hydrogen).

The gas is intensely poisonous, experiments having demonstrated a fatal effect on animals when the air contains but 0·25 per thousand of the gas.

To test for the presence of the gas, air may be aspirated over filter-papers, one moistened with a solution of nitrate of silver and the other with a solution of the acetate of lead. If PH_3 is present, the nitrate of silver filter-paper is darkened, but not the acetate of lead paper; while SH_2 darkens both papers. It is, however, preferable to separate any SH_2 before testing the action of the air on the $AgNO_3$ paper. The amount of PH_3 may be calculated by estimating the silver which is thereby precipitated from a standard solution of $AgNO_3$ ($3Ag = PH_3$).

Along with PH_3 a relatively small amount of AsH_3 may be liberated from ferro-silicon, and thus this equally poisonous gas may also gain access to the atmosphere.

TABLE OF THE AMOUNTS OF VARIOUS GASEOUS IMPURITIES WHICH HAVE BEEN SHOWN TO INJURIOUSLY AFFECT HUMAN BEINGS AND THE LOWER ANIMALS.

(*Compiled from the Investigations of Lehmann, Matt, Gruber, Ogata, Friedländer, etc.*)

Gas	Amount
Chlorine Bromine Carbon bisulphide Iodine	0·005 per 1,000.
Phosphuretted hydrogen Hydrochloric acid Sulph[illegible] acid [illegible]	0·01—0·05 per 1,000.
Ammonia Sulphuretted hydrogen Carbon monoxide	0·2—0·3
Carbonic acid	30—50 „

CHAPTER V

OZONE—PEROXIDE OF HYDROGEN

OZONE (O_3).

THIS gas is an allotropic oxygen, in which the molecule contains 3 atoms of oxygen instead of the 2 present in ordinary atmospheric oxygen. It is a gas with a peculiar phosphorous odour, and possessing marked irritating properties upon the mucous membrane of the eyes and nose and upon the respiratory tract. In nature it oxidizes oxidizable matter, and thus purifies air. It is best prepared artificially by passing electrical discharges through moist air, and hence it will be readily understood that it exists naturally in greatest quantities during and after thunderstorms, when it is also generally associated with nitric and nitrous acids and peroxide of hydrogen. The peroxide of hydrogen is also a powerful oxidizing agent, by parting with some of its oxygen and becoming water ($H_2O_2 = H_2O + O$). Nitrous acid also parts with its oxygen with great readiness.

There are good grounds for doubting whether ozone ever exists in air in appreciable quantity, and whether it ever exceeds 1 part in 700,000. Certainly most of the observations of ozone hitherto recorded have included peroxide of hydrogen.

According to Tidy—

1. Most ozone is found after thunderstorms, and least in damp and foggy conditions of the atmosphere.

2. More is found on the coast than inland, especially when sea-breezes are blowing.

3. More is found at high than at low levels.

4. More is found in country than in town districts.

5. More is found in winter (especially after heavy snowstorms) than in summer.

6. More is found during the night than the day, and most at dawn.

7. The western winds in Great Britain contain more ozone than the eastern. Houzeau points out that the manifestation of ozone upon ozone papers is affected chiefly by the *intensity* of the winds in most cases, except where these blow directly off the ocean.

8. It is rarely, if ever, found in the air of occupied dwelling-rooms.

Test.—A test for ozone which has been much employed is that of exposing to the atmosphere a white porous paper (filtering or blotting) previously soaked in a solution of potassium iodide and starch, and allowed to dry. Ozone will free the iodine, which then combines with the starch to form the blue iodide of starch, and

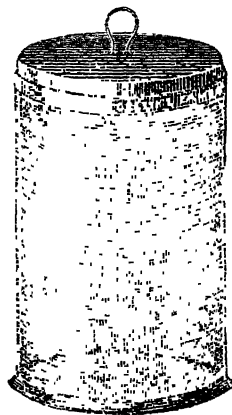



FIG. 28.—THE OZONE CAGE.

thus a blue colour is created ($O_3 + 2KI + H_2O = 2KHO + I_2 + O_2$). The papers are exposed in a cage, and observations are taken at least every twelve hours. The cage aids in protecting the papers from direct sunlight, dust, and rain, each of which may lead to a subsequent fading of the colour; it consists (Fig. 28) of a double cylinder of very fine wire gauze; and projecting downwards from the under part of the lid is a small hook, to which the ozone papers are attached.

The above-mentioned papers lead to errors of estimation from the following causes:

1. Nitrous oxide, peroxide of hydrogen and chlorine (each of which may also be present from electrical discharges in the atmosphere), and some volatile organic acids, produce similar

results upon the papers, and sulphurous acid and sulphuretted hydrogen tend to destroy the blue colour.

2. The freed iodine is partially volatilized, and thus its effect is lost, while some of it may return to the potash and form inert iodide and iodate.

3. It is impossible to get uniform conditions—*i.e.*, the amount of light, moisture, temperature, and wind varies, and makes results incomparable; and the purity and strength of the starch vary.

A better test (Houzeau) is the blueing of faintly reddened litmus-paper previously moistened with a 1 per cent. solution of potassium iodide and dried, when the ozone liberates the iodine, and the alkaline potash formed gives the paper a blue tint. In the absence of hydrogen peroxide, ammonia is the only other gas in the atmosphere which can produce the same effect, and, consebuently, another piece of the litmus-paper, *not* treated with potassium iodide, is exposed at the same time. Then any difference in the shades of the two papers *must* be furnished by ozone, which can be estimated by means of the ozonometer.

Perhaps the best papers for general use are those saturated with a mixture of 15 per cent. solution of KI, and a sufficient quantity of a 1 per cent. alcoholic solution of phenolphthalein to render the liquid opalescent. These papers are coloured a fugitive red with ozone, while chlorine, bromine, or nitrous acid only gives a blue or brown coloration (Arnold and Mentzel).

Ozone papers must be kept preserved from the air in a tightly closed bottle, for to air containing the merest trace of ozone the papers react. But immediately before using them for test purposes they should be moistened, and the tint matched as quickly after the test as possible. Hydrogen peroxide reacts similarly to ozone upon all these papers, and any such ozone estimations are, in consequence, vitiated by this gas.

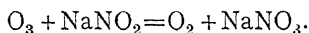
Schöne has pointed out that ozone blackens a bright piece of silver foil, but hydrogen peroxide has no such effect. Moreover, chromic acid, whether in the solid form or in solution, decomposes even the most dilute peroxide of hydrogen, while it has no action on ozone.

Engler and Wild find that the best test for ozone is by means of the chloride of manganese, which not only yields an extremely delicate reaction, but, in consequence of its hygroscopic character, keeps the prepared paper of the requisite moisture. Ozone turns

such paper brown by the formation of manganese dioxide. Hydrogen peroxide and nitrous acid have no such effect, but since ammonia and its carbonate turn these papers brown, the colour should be further tested by moistening with tincture of guaiacum, when, if the papers have been acted on by ozone, a blue colour will be developed even before the brown has had time to disappear; whereas no blue forms if the browning is due to ammonia.

The method by which a sufficiently approximate quantitative estimation of ozone (*i.e.*, "ozonometry") is usually made is colorimetric. The intensity of the colour created by the ozone when the prepared papers are exposed to the atmosphere, generally for two hours, is matched against a standard scale (1 to 10) of tints, each tint having been originally produced by exposing similar papers to *known* amounts of ozone. The greater the movement of the air, the greater the quantity brought to act upon the paper, and hence less quantities of ozone present in the atmosphere on windy days may create more colour than greater quantities on still days. The only way, therefore, by which an accurate comparison of the ozone in different atmospheres can be made is by lining a dry glass tube with the ozone papers, and then aspirating similar quantities of air through such tubes.

F. L. Usher and B. S. Rao have devised a useful method for the estimation of ozone and nitrogen peroxide in small quantities in the air. In principle the method is extremely simple, and depends on the reaction between ozone and alkali nitrite in aqueous solution, a reaction which they have found to take place quantitatively according to the equation:

$$O_3 + NaNO_2 = O_2 + NaNO_3.$$

Two samples of air are taken and collected in 7-litre stoppered bottles. One sample is admitted through two tubes containing reepectively chromic acid and powdered manganese dioxide, and the other through a tube containing chromic acid only. The samples thus collected are shaken with a dilute standard solution of sodium nitrite made slightly alkaline, and the nitrite content of the bottles is subsequently determined colorimetrically. The first sample of air contains only nitrogen peroxide, the ozone and hydrogen peroxide having been destroyed, and the increase in the quantity of nitrite in the bottle is equivalent to the nitrogen peroxide absorbed. The second sample contains ozone

and nitrogen peroxide, and the difference between the quantities of nitrite in the two bottles after shaking is equivalent to the ozone present.

Peroxide of Hydrogen.

H_2O_2 is generally present in traces, but it exists in considerable quantities during and after thunderstorms. It has been seen that it has similar properties to those of ozone; it may be distinguished, however, by certain of the tests given under "ozone," and also by the fact that it is only after the lapse of several hours that it reddens potassium iodide paste. A good test for the presence of hydrogen peroxide is the following: To some distilled water that has been made to take up the vapour add a drop of a 1 per cent. solution of potassium chromate, followed by a little ether and a few drops of dilute sulphuric acid. On shaking, the ether takes up the blue colour of perchromic acid. The test is fairly delicate.

CHAPTER VI

SUSPENDED MATTER IN THE AIR

THE nature of the suspended matter found in the atmosphere must necessarily vary widely with the place and the circumstances of its collection; and it would not be going too far to say that particles of almost everything the observer can see about him may be included. Obviously, the amount increases according to the extent to which the atmosphere departs from its state of greatest purity, high mountain air on the one hand containing few, and low town air containing many.

It is more especially in factories and workshops that the examination of suspended matters is important, since both the nature and number of the particles have been shown to determine the prevalence of lung disease. These minute particles have a tendency to settle when the air is still, and the collection and microscopical examination of the dust which settles in a closed room furnishes a rough means of qualitative examination.

The Methods of Collection:

Undoubtedly the *best method* is to aspirate large volumes (100 litres) of air slowly through small amounts (100 c.c.) of distilled water, placed in one or two small wash-bottles; the bottles are then connected together and with the aspirator by rubber tubing, as shown in Fig. 26. The waters may then be mixed and evaporated to about 20 c.c., when drops may be mounted and examined by the microscope.

This method can be made a quantitative one by aspirating a measured quantity of air through one or two wash-bottles, mixing the waters, and then counting the number of particles in an aliquot part; or the water may be evaporated to dryness in a weighed platinum dish, and the weight of residue collected will be the weight of suspended matter in the volume of air aspirated, and after ascertaining the loss on ignition, this may also be

expressed as "volatile" and "non-volatile." This method is the most suitable for collecting trade dusts. If lead dust is so collected the lead may be dissolved out from the ignited total solids by nitric and hydrochloric acids, diluted, and then estimated colorimetrically. The results should be expressed in terms of milligrammes per cubic metre.

Another method is by means of *Pouchet's aëroscope.*

This instrument consists of a vertical glass cylinder, capable of being hermetically closed at either end by a copper ferrule. The ferrule at the upper extremity of the cylinder is a permanent fixture, and gives passage to a vertical copper tube which is partly outside and partly enclosed within the cylinder; of this tube, the extremity of the part which is outside the cylinder is expanded into a trumpet-shaped mouth, and the end of the part which is inside the cylinder is gradually drawn to a very fine point, not more than 0·5 millimetre in diameter.

The ferrule at the lower extremity of the cylinder is temporarily removed, so that a circular glass slide—which has been previously smeared with pure clean glycerine—can be placed with its centre immediately under the finely drawn point of the copper tube. The whole apparatus is then made air-tight and connected with the aspirator. The air which is thus sucked in falls in a spray upon the glass slide, and the glycerine retains the suspended matter. Subsequently the slide can be removed and examined by the microscope.

A slight modification of Pouchet's aëroscope is the instrument of Marié Davy. The accompanying figure sufficiently explains itself.

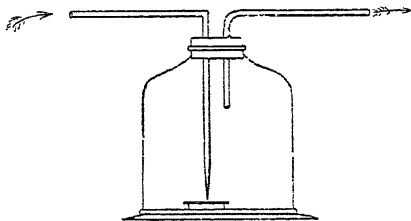



FIG. 29.—M. MARIÉ DAVY'S MODIFICATION OF POUCHET'S AEROSCOPE.

Hesse's apparatus is seen by Fig. 30 to consist of a long glass tube connected at one end to the aspirator; the small india-rubber cap which closes the other end is removed just before use, and 50 c.c. of pure glycerine is poured into the tube, which is then

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turned round so as to make the glycerine coat the whole interior. As the air is subsequently aspirated through the tube, the suspended matter is caught up by the glycerine, which can be removed by a clean spatula and examined microscopically.

But methods in which glycerine is employed arė somewhat unsatisfactory, for the reason that the original glycerine will generally contain solid particles. A preliminary microscopic examination of the glycerine, however, does not entail much additional labour or time, and thereby the nature and amount of the foreign matter it contains can be previously noted.

A *fourth plan* entails the use of a pure sugar filter through

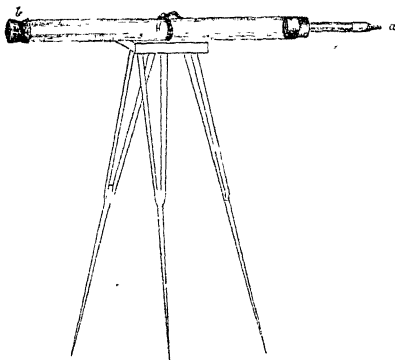



FIG. 30.—HESSE'S APPARATUS FOR THE COLLECTION OF SUSPENDED MATTERS IN THE AIR.

a, The extremity connected with the aspirator; *b*, a removable cap.

which the air is slowly drawn; the sugar is then dissolved in a sufficiency of pure water, when the suspended matters caught up in it are retained in suspension in the water, and may be collected and examined microscopically. The filter is best arranged as a glass tube, at least an inch in diameter, disposed horizontally, and packed (not too tightly) for several inches with the sugar crystals. One end of the tube is left open for the entrance of air, and the other connected by india-rubber tubing with an aspirator. The filter dissolved, the suspended matter may also be separated by filtration through a weighed Swedish filter-paper, then thoroughly washed and dried at a low temperature and weighed. If the amount of air aspirated has been

measured, the weighed quantity of its original suspended matter can be expressed quantitatively.

It is obvious that the amount of the dust in town air must vary considerably; commonly the extent of this variation is between 5 and 25 milligrammes per cubic metre; it has been estimated as high as 224 in cement works during work.

J. Aitken has devised an ingenious method of enumerating the particles of suspended matter in the atmosphere. A full description of the elaborate apparatus may be studied in the Proceedings of the Royal Society of Edinburgh, 1889. In this method a measured quantity of air is taken and passed into a receiver, where it is mixed with a large measured quantity of filtered (dustless) air, and saturated with water. The air in the receiver is then expanded by means of an air-pump; a shower of rain is thus produced which carries down the suspended matter, and the number of particles which fall on a measured area are then counted, it being assumed that each drop has for its nucleus a dust particle. From 10,000 particles per c.c. of air to over 2,000,000 may thus be obtained from the outside air, and in occupied rooms near the ceiling they may reach many millions. In the centre of London the average is from about 250,000 to 500,000 per c.c., while at the top of lofty mountains and in mid-ocean the dust particles may number only from 200 to 300.

Aitken has demonstrated that it is the dust in the atmosphere which determines mists and fogs, inasmuch as the condensation of aqueous vapour is not determined only by reduction of temperature, but also by the presence of particles of dust, each particle becoming shrouded with a covering of moisture. By his method of enumeration it is assumed that each droplet has for its nucleus a dust particle.

Vörner employs a blackened resinous substance which on cooling presents a uniform and polished surface. This surface may be protected from dust access prior to the experiment by means of a watch-glass sealed down with vaseline. For the purpose of an experiment the watch-glass is removed and an exposure of ten minutes is made; when, by means of an electric lamp, the number of dust particles per square centimetre may be counted.

A measured volume of air may be drawn through filter-paper and the degree of discoloration produced on the paper compared

with a calibrated scale. Or the air may be drawn through the paper until a predetermined degree of discoloration has been produced, the volume required being measured. Such an instrument has now been in use for some time in Glasgow, and enables a rapid measurement of the degree of pollution by suspended matter to be made. Briefly, it consists of two bottles used as aspirators; water allowed to siphon from one to the other draws a measured volume of air through a disc of filter-paper of standard size, the discoloration of which is compared with a scale of shades. An observation can be taken in about ten minutes.

Dust collected from the top of a wardrobe in a bedroom yielded the following results on analysis:

Moisture	4·4
Organic matter	52·6
Silica and insoluble silicates	21·0
Oxide of iron and alumina	9·7
Lime (CaO)	6·2
Carbonic acid, with traces of sulphuric and phosphoric acid	6·1
	100·0

The inorganic matter was mostly amorphous, while the organic was for the most part organized. Among the commonest constituents of the latter were vegetable and animal fibres derived from fabrics such as linen, cotton, and wool. In addition, there were a few feather barbs and particles of carbon; squamous epithelial cells from the skin, starch granules, and a few pollen spores were also identified.

The dust from 20 square yards of glass roofs at Kew and Chelsea was found to contain nearly 5 per cent. of SO_3, equal to about 2 per cent. of S.

The soot-fall from the atmosphere over industrial centres can be estimated by collecting it in a hopper of known collecting area, which terminates below in a small tube connected with a capacious bottle. The apparatus is similar to a rain-gauge, and it collects, of course, both rainfall and deposit. All rain and deposit matter falling on such a gauge vessel may be collected, and the total solid matter weighed and analyzed. *The insoluble matter* consists largely of road dust and carbon. The portion

soluble in carbon bisulphide is largely composed of tarry matter. This tarry matter attaches itself to the carbon particles, and gives them very great adhesive power, so that when they have deposited on and attached themselves to building-stones, plants, etc., they cannot be readily washed away by rain, as would be the case if the tar were not there.

The "loss on ignition" of the undissolved matter represents largely the carbonaceous matter, such as soot, emitted in the form of smoke. It would also represent any organic matter of vegetable or animal origin carried into the air in the form of dust. A further, but relatively small, portion consists of water of constitution from clayey matter, carbon dioxide from limestone, and the like.

The *dissolved matter* consists of that portion of the total solids which is readily soluble in water, such as chlorides, sulphates, and other salts. Free acids originally present in the atmosphere, such as sulphur dioxide or sulphuric acid, may react with the basic constituents of the undissolved matter, and go into solution either in the course of, or after, deposition.

The sulphates present in the liquid portion are estimated and returned in terms of SO_3—*i.e.*, sulphur trioxide or sulphuric anhydride—which represents the acid portion of all sulphates. The basic portion, such as ammonia, soda, lime, alumina, need not be included. The SO_3 can be taken to represent the oxides of sulphur derived from the combustion of coal, oil, or gas containing sulphur of any kind.

It has been found that in the city area of London some 650 tons of soot fall upon every square mile each year. There are marked differences between domestic and boiler soot. The latter is little more than dust or ash, practically all the hydrocarbons having been burnt; whereas the former possesses a high content of tar and volatile substances and a low content of ash.

The Nature of the Suspended Matter of Air.

The following substances may be found:

Animal.—Débris from wear and tear of clothes, etc.; wool and silk fibres; human hair; particles of feather; débris of dried epithelial cells, and epidermic scales from skin; fragments of insects—*i.e.*, scales from wings, legs; particles

of the spider's web; dried fæcal particles from horses' dejecta; minute ova; amœbiform organisms; molecular débris in considerable quantity.

Vegetable.—Particles of carbonaceous matter ("soot"); molecular débris in large quantity; vegetable fibres, hairs and cells; cotton and linen fibres; starch grains; portions of plants, and pieces of woody fibre; pulverized straw; moulds, fungi, diatoms, and bacteria and their spores; pollen grains; algæ, notably *Protococcus pluvialis* and also the small oval cells of other unicellular algæ. The spores and mycelium of *Achorion Schönleinii* and *Tricophyton tonsurans* have been found in the atmosphere of skin wards.

Mineral.—Especially numerous when the ground is dry. Minute particles of every chemical constituent of the soil may be raised up into the atmosphere—*e.g.*, silica, silicate of alumina, chalk, peroxide of iron, etc. Sodium chloride is invariably present, and is in greatest quantities at the seaside. Lead, arsenic, and zinc may be furnished by the wall-papers, paint, and "dryers" employed upon the walls of rooms; arsenic also from artificial fruit, flowers, curtains, etc., used for ornamentation; coal dust, etc.

There are certain trade dusts which vitiate the air of the immediate neighbourhood in which the trade processes are carried on; and particles of a great variety of substances may thus find their way into the atmosphere from coal, tin, stone, slate, cement, wood, clay, steel, flour, textile fabrics, glass, etc.; while poisonous matter may get into the atmosphere where lead, arsenic, copper, chromium, phosphorus, and mercury are being used for trade purposes.

CHAPTER VII

THE CHARACTERS OF THE AIR COLLECTED FROM VARIOUS SOURCES—BACTERIOLOGICAL NOTE

MARSH AIR.

THE air collected over marshy regions is contaminated by the products of vegetable decomposition.

Such air contains excess of carbonic acid, commonly 0·05 per cent.; marsh gas may be markedly present; sulphuretted hydrogen is also sometimes appreciable; watery vapour in large amount; ammonia in traces; phosphuretted hydrogen in faint trace. The suspended matter is found to mainly consist of vegetable débris; algæ, diatoms, fungi, and other micro-organisms.

In many cases where the presence of sulphuretted hydrogen is appreciable the marshy waters contain soluble sulphates, which become deoxidized to sulphides by reducing agents (chiefly organic matter), and the sulphuretted hydrogen doubtless results from the action of vegetable acids upon these sulphides.

SEWER AIR.

Sewer air varies in composition with the sewage and the state of the sewerage system. Its reaction is generally alkaline. Its temperature practically never falls below 9° C., and it is always saturated with moisture, or nearly so.

Oxygen is variously diminished, according to the efficiency of the sewer ventilation; it is sometimes in normal proportions.

Carbonic acid is variously increased from the same cause; it probably does not average in a good modern system of sewerage more than three times the amount normal to the atmosphere, but it may be ten times as great, or even more.

Ammonia is somewhat in excess of the external air, and it may be greatly in excess.

Sulphuretted hydrogen, Ammonium sulphide, Carbon bisulphide } may be present in variable quantities, but usually only in traces. If, however, the sewage stagnates in the sewer, the sulphuretted hydrogen may be present in considerable amount. Marsh gas is in traces, or absent; and traces of nitrous dioxide and phosphuretted hydrogen may be present.

The fœtid and putrid organic vapours of sewage are, according to Odling, allied to the compound ammonias, and are probably carbo-ammoniacal and contain traces of animal alkaloidal substances.

The odour of sewer air is not usually due to sulphuretted hydrogen, but to minute quantities of a variety of volatile substances, such as indol, skatol, the mercaptans, and compound ammonias.

The micro-organisms are almost exclusively moulds and micrococci, and these, together with animal and vegetable débris, appear to constitute the very sparse suspended matter.

The micro-organisms in the sewer air are related more to the micro-organisms in the air outside than to those of the sewage (Andrewes and Law); they are generally fewer in number than those of the outside air at the same time. Splashing may, however, disseminate sewage bacteria in sewer air, and possibly also the bursting of bubbles or the ejection of minute droplets from flowing sewage (Haldane, Carnelly, Horrocks, Andrewes).

The organic matter in sewer air probably averages from two to three times the amount in the outside air.

The Air in Coal Mines.

The oxygen is diminished, and the reduction may be extremely faint or so considerable that the total oxygen does not much exceed 18 per cent. The carbonic acid is increased, and may reach 1·5 per cent. or over. A faint trace of carbon monoxide is generally present. The considerable variations in the amounts of oxygen and carbonic acid in different mines are mainly dependent upon the ventilation provided. Marsh gas is sometimes in large amount, but it may be only in traces in some mines. A little sulphuretted hydrogen may be present, and in some mines high percentages of "black damp" or "choke damp" get into the atmosphere. "Black damp" is a mixture of nitrogen with a relatively small proportion (generally from 10 to 15 per cent.) of carbonic acid.

The marsh gas, or methane, may be estimated in the following manner: The volume of carbonic acid present in a sample of the air is first determined by absorption in a 10 per cent. solution of caustic potash, then the methane is burnt by passing the air to and fro over a spiral of incandescent platinum wire. The contraction resulting from the combustion of methane is exactly double the CO_2 formed. Methane on combustion produces its own volume of carbonic acid; and the carbonic acid so produced may be absorbed in the solution of caustic potash, and its volume thus measured. The contraction on combustion and the volume of carbonic acid formed would bear a different ratio to one another if the combustible gas were any other than methane.

For useful particulars of mine air analyses the reader should consult "Methods of Air Analyses," by J. S. Haldane, M.D., F.R.S.

Town Air during Fogs.

Reaction acid; oxygen is slightly diminished; carbonic acid very much increased—may even exceed 0·09 per cent.; sulphurous and sulphuric acids markedly present; carbon bisulphide in traces; maybe carbonic oxide, ammonia, ammonium sulphide or carbonate in traces; sulphuretted hydrogen generally in faint traces; watery vapour excessive; fine suspended particles of carbon and tarry matters, together with an increase of the commoner form of suspended matter in air.

Ground Air.

Ground air may be drawn from considerable distances into a house, especially during periods of frost, owing to the aspirating effect of the warmed and expanded air of the house itself; and the foul air of a leaky drain or cesspool may, under favourable circumstances, be sucked through the earth into a dwelling for a distance of many yards. When it is borne in mind that many houses contain cellars built and ventilated considerably below the ground level, it will be realized that "ground air" must enter materially into the constitution of the atmosphere of such cellars.

Ground air contains an enormously high percentage of carbonic acid, and the maximum amount of this impurity is always found between July and November, when the prevalent temperature and moisture favour the rapid decomposition of

vegetable matter. The ground air of sandy soils contains relatively little CO_2.

Ground air commonly contains traces of ammonia, sulphuretted hydrogen, and hydrocarbons. It is very free from micro-organisms. The CO_2 increases with the depth of the soil, and it is sometimes as much as 5 per cent. in deep soil a few feet from the surface.

The entrance of ground air into ground-floor rooms, basements, and cellars may be detected by comparing the carbonic acid found in these rooms with that in the external atmosphere, when any considerable excess of this gas (not otherwise accounted for) points to such pollution. The source of other impurities

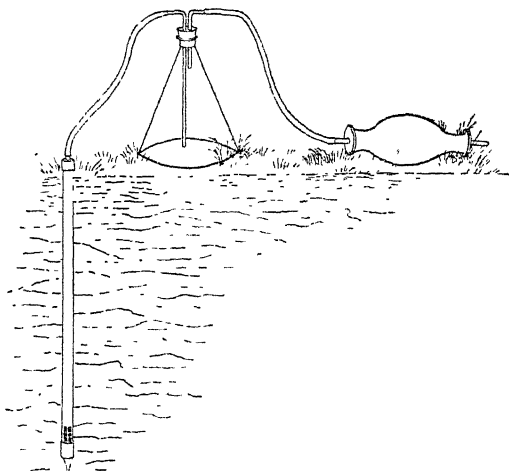



FIG. 31.—HESSE'S APPARATUS FOR COLLECTING GROUND AIR.

present may also be traced by collecting samples of the ground air in the vicinity of the house and comparing the results of such examination.

A sample of ground air may be conveniently taken in the following manner: A sharp-pointed narrow steel cylinder, with numerous perforations just above its point, is driven into the earth to depths varying from 1 to 4 feet. The upper end of the tube is connected with a large air-jar, which is again connected to an aspirator. The connection between the jar and the steel cylinder is shut off, and the jar is first emptied (by means of the aspirator) of the air it contains; the connection is then re-established and the sample collected by aspiration.

Bacteriological Note.

Bacteria are always present in air, but, unless the air contains a large number of dust particles, in comparatively scanty numbers.

The determination of the number of organisms is of greatest use as a means of comparing methods of ventilation. Pathogenic organisms are not readily detected in air, the organisms usually found being moulds and saprophytic bacteria.

Flügge has shown that in inhabited rooms the air is liable to be contaminated with bacteria derived from the presence of droplets of mucus extruded from the buccal cavity in the acts of sneezing, coughing, and loud speaking.

Gordon has greatly extended our knowledge of such particulate pollution, and has shown that certain bacteria, which can be detected and estimated, furnish means whereby these different kinds of pollution can be recognized.

According to Gordon, pollution of three separate kinds can be recognized by bacterial tests.

1. *Pollution from Material derived from the Upper Respiratory Passages.*—Gordon has shown* that certain streptococci are present in enormous numbers in human saliva, and that their presence serves as a means whereby the addition of saliva to air can be detected. The organism specially characteristic of such pollution is the *Streptococcus salivarius.*

2. *Pollution from Material detached from the Skin.*—The *Staphylococcus epidermidis albus* is constantly present on the human skin, and by its detection in air the presence of particles detached from the skin may be deduced.

3. *Pollution by Material brought in from the Street on Boots.*—Such material consists largely of horse-dung, and may be recognized by the presence of *B. coli,* spores of *B. enteritidis sporogenes,* and *Streptococcus equinus.*

* Local Government Board, Medical Officer's Report, 1902-03, p. 4.

CHAPTER VIII

SCHEME FOR THE DETECTION OF GASES WHEN PRESENT IN LARGE QUANTITIES

WHEREAS for the detection of various gases which may contaminate the atmosphere it is necessary to pass large volumes of air through distilled ammonia-free water containing agents with which the gases will combine, and then to apply the necessary tests, yet, when these gases exist in considerable quantities (as in the atmosphere of chemical manufactories, or those manufactories in which chemicals are employed), they may often be discovered by tests applied to small quantities of the air collected in air-jars.

1. Moisten two pieces of delicate red and blue litmus-paper in neutral distilled water, and catch these between the stopper and the neck of the bottle in such a way that they hang down into the bottles free of the sides. Note any change in the colour of these papers after waiting two or three minutes.

2. If the reaction is acid or alkaline, pour rapidly into the jar a small quantity of distilled ammonia-free water (*i.e.*, about 10 c.c.), and replace the stopper at once; then shake vigorously, so that some of the gas may be taken up by the water.

A. *If the blue litmus-paper turns red* (*i.e.*, the gas is acid), it is either carbonic acid, hydrochloric acid, sulphurous acid, nitric or nitrous acid.

Add a drop or two of a solution of silver nitrate to some of the water poured from the air-jar into a test-tube.

(*a*) *A white precipitate* denotes the presence of either—

1. *Carbonic Acid.*—*Very slight* precipitate, insoluble in nitric acid; acidity also *very faint*. Clear baryta water added to the jar becomes turbid after shaking, and the turbidity is increased by adding ammonia.

2. *Hydrochloric Acid.*—*Marked* precipitate, insoluble in nitric acid, but soluble in ammonia and potassium cyanide; acidity also *marked*.
3. *Sulphurous Acid.*—*Marked* precipitate, soluble in nitric acid; the precipitate on being heated clears up and the solution darkens (Ag_2S). The water from the jar will decolorize iodide of starch solution; and if it be warmed after the addition of hydrochloric acid and zinc, a piece of lead acetate paper held over the test-tube becomes darkened ($SO_2 + 3H_2 = SH_2 + 2H_2O$). Odour characteristic.

(*b*) *There is no precipitate*, and this fact denotes the presence of either—

1. *Nitric Acid.*—Add brucine and sulphuric acid to some of the water from the jar, and note the appearance of the pink zone changing to yellow and brown; or add a crystal of ferrous sulphate and then sulphuric acid to the water, and note the brown coating of the green crystal.
2. *Nitrous Acid.*—Add a drop of a solution of starch and potassium iodide, and then a drop of sulphuric acid. A blue colour *forming at once* denotes the presence of this acid; or the Ilosvay test may be applied.

B. *If the red paper is turned blue* (*i.e.*, the gas is alkaline), it is either—

1. *Ammonia.*—Add a drop or two of Nessler's reagent to a little of the water from the jar, when a yellow to orange colour appears. Odour characteristic.
2. *Ammonium Sulphide.*—Nessler's reagent causes a black colour to appear when added to some of the water from the jar; and a solution of nitro-prusside of sodium produces a violet colour. Odour characteristic—*i.e.*, that of rotten egg predominates, but it is easy also to detect the presence of ammonia.

C. *If the litmus is not affected* (*i.e.*, the gas is apparently neutral), it may be—

Sulphuretted Hydrogen.—Lead acetate papers placed in the jar are darkened, as are also solutions of lead, iron, or copper salts. Odour characteristic.

D. *If the litmus-papers are first reddened and then slowly bleached,* the gas is—

Chlorine.—Filtering paper moistened in a solution of potassium iodide and suspended in the jar is first darkened by liberated iodine and then bleached. Odour characteristic. Furnishes a red colour with a mixture of sulphocyanide of potassium and a proto-salt of iron.

Note.—Sulphuretted hydrogen has many reactions in common with ammonium sulphide—*e.g.*, both gases will darken lead acetate papers and solutions of lead, copper, or iron salts, and their odours are closely similar; but they may be readily distinguished if attention is paid to the subjoined differences:

Ammonium Sulphide.—Alkaline reaction; produces a violet colour with a solution of nitro-prusside of sodium; odour of rotten eggs and ammonia.

Sulphuretted Hydrogen.—Neutral reaction; no effect upon the nitro-prusside; odour of rotten eggs alone.

PART V

FOOD EXAMINATION

CHAPTER I

MILK

THE COMPOSITION OF COW'S MILK AND OF OTHER MILK.

MILK consists of water, proteids, milk-sugar ($C_{12}H_{22}O_{11}$), and mineral salts (chiefly phosphates of calcium. magnesium, and potassium, and chlorides of sodium and potassium; very small quantities of sulphates are present, and traces only of carbonates, if any).

The fat is suspended as minute globules, and since it forms the lightest element in the milk it tends to rise to the surface in the form of "cream," the largest globules being the first to separate. The milk early undergoes a souring, followed by a natural separation into a solid "curd" and a liquid "whey." This change is caused by the fermentative conversion of the milk-sugar into lactic acid by the agency of micro-organisms which gain access to the milk, the chief of which is known as *Bacillus acidus lacticus*. Acetic, succinic, and carbonic acids are also produced in small amounts.

The "curd" is found to consist of casein, albumin, and traces of other kindred nitrogenous substances, the "whey," of the water, milk-sugar, and salts.

A still later change is characterized by the appearance of a bluish tint, which is ascribed to the growth of another micro-organism, called *Bacillus syncyanus*; and ultimately the casein decomposes owing to the access and development of putrefactive bateria.

The *average composition* of pure cow's milk is as follows:

Water, 87·40.

Solids, 12·60, consisting of:
- Sugar, 4·75.
- Fat, 3·65.
- Protein, 3·48.
- Mineral salts, 0·72.

Vieth's ratio of the sugar, proteids, and ash in cow's milk is 13 : 9 : 2.

The milk of individual cows, collected under circumstances which preclude the possibility of any sophistication, has, however, been found to vary considerably in its composition.

There is no parallelism between rainfall and composition of cow's milk; and from experiments undertaken by the Board of Agriculture it appears that the excessive drinking of water by cows has no direct bearing on the composition of their yield.

The circumstances which mainly determine the variations are:

(*a*) The breed of the cow. Alderneys give most fat, and Longhorns most casein.

(*b*) The time which has elapsed since the last milking. The longer the interval between the milkings the poorer the quality. Richmond finds that evening samples generally show from 0·3 to 0·4 per cent. more fat than morning samples.

(*c*) The stage of milking. That which is first drawn ("fore-milk") contains very little cream (under 0·5 per cent.); but towards the end of the milking the cream is very high in amount; and the very last quantities drawn from the udder ("the strippings") are almost pure cream.

(*d*) The health of the animal.

(*e*) The age; young cows secreting milk of a poorer quality.

(*f*) The time of year. The lowest fat occurs in April, May, and June, and the highest in October; and during July and August the solids-non-fat are below the average.

(*g*) The period which has elapsed since last calving affecting the presence or absence of colostrum and the richness of the milk. The total solids (especially the fat) increase with the advance of the lactation period.

(*h*) The food taken; beet and carrots throw up the sugar.

Where the amount of fat is very high the solids-non-fat are frequently below the average in pure milk, the deficiency being due to deficiency in milk-sugar and not to proteids or ash; and

a cow yielding a high figure of fat in the afternoon may give below the average in the morning.

It is very rare, however, that in dairy samples containing the mixed milk of several cows the non-fatty solids fall below 8·5 per cent., or the fat below 3 per cent.

The following table (after D. Richmond) shows a comparison between the milk of various animals:

	Water.	Casein, Albumin, etc	Fat.	Sugar.	Salts.
Human	87·80	2·20	3·30	6·40	0·30
Cow	87·20	3·57	3·76	4·75	0·72
Ewe	79·46	6·68	8·63	4·28	0·97
Goat	86·04	4·35	4·63	4·22	0·76
Mare	89·80	1·84	1·17	6·89	0·30
Ass	90·12	1·66	1·26	6·50	0·46

It will be seen from this table that the proteid material varies from 2·20 in human milk to 6·68 in the ewe; that the fat is lowest in the mare's milk (1·17), and highest in the ewe's (8·63); that sugar ranges from 4·22 in the goat to 6·89 in the mare; and that the water is least in amount in the ewe's milk (79·46), and greatest in that of the ass (90·12).

Thus, adopting the cow's milk as a standard for comparison, *human milk* (though varying greatly with the period of lactation, etc.) shows an increased quantity of sugar and a slightly increased quantity of water, but all solid constituents, with the exception of sugar, are less, the total protein amounting to barely two-thirds. *Mare's milk* is also richer in sugar and water; but the fat, casein, albumin, and ash are considerably less. *Goat's milk* is richer in the solid constituents except sugar, and therefore contains a less percentage of water. *Ewe's milk* is characterized by the very high amount of fat, casein, and albumin; the ash is higher than in cow's milk, but the sugar and water are less.

Citric acid is a normal constituent, in minute quantity, of cow's milk and of human milk.

The Milk of Diseased Cows.

Although the milk secretion is in abeyance during some diseases, it is not so in all, nor is it so in all cases of the same disease. In a few conditions the milk presents somewhat definite chemical

and microscopical characters; to the naked eye it may be all that is desired.

It is in cattle plague and foot and mouth disease that the changes are most marked.

In cattle plague the sugar is markedly diminished; the fat is increased, together with—to a less extent—the casein and salts (Gamgee). Blood and pus are also commonly detected in the milk.

In foot and mouth disease the milk commonly contains pus, blood, or mucus (*i.e.*, in those cases where there is ulceration of the teats or abscesses in the udder); and, as in cattle plague, the milk corpuscles are seen under the microscope to display a tendency to aggregate into grape-like clusters. When the disease is advanced, bodies resembling pus cells (though a little larger), and large yellow granular bodies, together with pus and blood-cells, are also present. In this disease the results of chemical analyses vary so considerably as to be of no value for diagnostic purposes; but the milk separates remarkably quickly on the application of a gentle heat into curds and a pale blue whey; and this feature alone is considered as almost diagnostic by some continental observers.

In *tuberculosis* the milk is not at first appreciably affected, but the fat, lactose, and casein diminish toward the later stages of the disease.

In *garget* the milk from the inflamed quarters of the udder is often thin and poor in solid constituents, and blood and pus may be present.

CHAPTER II

THE ANALYSIS OF MILK

The Physical Characters:

1. *Consistence.*—The milk should be quite opaque when placed in a narrow glass tube; otherwise it has probably been watered, and has a bluish tint.

Sometimes it is thick and viscid ("ropy"), and on pouring has an appearance somewhat akin to mucus. Such milk has the property of imparting, when added in small quantities, its own peculiar quality to large bulks of good milk. This condition may be due to inflammation of the udder or to the growth of certain micro-organisms (*B. mesentericus* or similar organisms), whereby a mucinous substance results from changes in the milk-sugar and casein. It is usually accompanied by considerable acidification.

"Colostrum" (the milk yielded during the first few days after the birth of the calf) coagulates on heating, owing to the larger quantity of albumin it contains; it is also more yellow than ordinary milk, shows flocculi, and has a slightly insipid saline taste. Some milks coagulate shortly after being drawn; these are, of course, very acid, and are generally yielded by cows in febrile conditions while suffering from inflammation of the udder.

2. *Colour.*—The colouring matter of milk (lactochrome) is in association with the fat globules, and to a slight extent with the casein; and as soon as the fat is separated in the form of cream, the original colour largely disappears. A good milk should be white, with the faintest possible suspicion of yellow, although such food as buttercups, carrots, mangel-wurzel, etc., tend to increase the yellow colour. A marked yellow may also occur naturally in milk containing considerable quantities of colostrum

corpuscles, where the animal is jaundiced, or where there are certain congestive conditions of the udder. The colour is also artificially furnished by dairymen by means of the addition of colouring agents.

Rarely, the freshly drawn milk is of a faint blue hue, or even green, or reddish. The cause of these colorations has been ascribed to the food consumed, and also to micro-organisms. Such milks have been known to cause severe gastro-intestinal irritation; and more especially is this the case with "blue" milk. When these colours form *slowly* after the milk has been drawn, it seems probable that they are due to micro-organisms (such as *B. cyanogenus*).

A pinkish hue is sometimes created by the presence of blood, but generally the blood tends to deposit.

3. *Taste and Odour.*—Milk has the power of absorbing any odorous gases with which it comes in contact, and of acquiring and retaining distinct flavours from the food consumed by the animal secreting it; thus cows which have been feeding upon turnips, garlic, fennel, damaged ensilage, distillery grain, etc., yield a milk which tastes of these articles; and when bitter medicines have been administered, or chestnut or vine leaves have been eaten, or when the cow suffers from some forms of liver disease, a bitter flavour is imparted to the milk. A bitter flavour may also be produced by certain micro-organisms.

4. *Reaction.*—This may be neutral or alkaline when freshly drawn from the udder, but the milk is commonly amphoteric in reaction, turning red litmus-paper blue and blue litmus-paper red. This amphoteric reaction with litmus results from the presence of two salts with opposite reactions. The acid reaction is due to the primary sodium phosphate (NaH_2PO_4), and the alkaline to the secondary phosphate (Na_2HPO_4). Milk is generally faintly acid by the time it comes to be analyzed; if markedly acid, lactic acid fermentation has well set in; and if markedly alkaline, some alkaline salt (such as sodium bicarbonate) may have been added.

The acidity may be calculated by running into 50 c.c. of the sample $\frac{N}{10}$ sodium hydrate (phenolphthalein being used as the indicator), each c.c. representing "1 degree" of acidity; this multiplied by 0·009 gives the percentage expressed as lactic acid (Richmond). (A degree of acidity represents 1 c.c. of N. acid to the litre, or 1 c.c. of $\frac{N}{10}$ acid to 100 c.c.) Milk as sold to the

consumer should not curdle when shaken up in a test-tube with an equal bulk of spirit containing 70 per cent. of alcohol by volume. On inclining the test-tube and bringing it back to the vertical position flakes or films adhere to the sides if the acidity is above 8 degrees.

5. *Sediment.*—Any foreign suspended matter is generally readily seen on the white background which the fluid itself presents; and any such matter may be detected at the bottom of the cream tube after the milk has stood in this for several hours. Dirt (cow-dung, dust, grit, hairs, textile fibres, pus, blood, epithelium, etc.) will deposit on standing, especially if the milk is well diluted with water.

6. *Dirt.*—The dirt in milk may be estimated by taking 100 c.c. of the sample, centrifugalizing this, and decanting the fluid portion. The sediment is then shaken with 15 c.c. of 10 per cent. ammonia, the mixture being diluted after the lapse of one hour with water and again centrifugalized. The opalescent liquid is then decanted, the sediment washed with water into a weighed platinum crucible, and further washed successively with alcohol and ether. The crucible and its contents are then dried at 100° C. until constant in weight (Fendler and Kuhn).

S. *Délépine's Souring Test of Cleanliness.*—Samples placed in test-tubes are incubated at 35° C. for fifteen hours, at the end of which the tubes are immersed in a bath of boiling water and left there for ten minutes.

On removal from this boiling water the contents of the tubes are examined. All the badly contaminated samples will be found clotted. The characters of the clots and the amount of gas given off (indicated by the amount of frothing) show within certain limits the degree of contamination.

After incubation at 35° C. for fifteen to twenty hours, and boiling for ten minutes, various degrees of pollution are indicated by the extent of these changes.

	Gas.	Clot.	Whey.
Very pure milk ..	No excess	None	None.
Slightly polluted milk	Abundant	Very small (milk thick)	None.
Polluted milk	Abundant	Large (milk solid)	None or scanty.
Heavily polluted milk	Abundant	Broken up (contracted)	Abundant.

Useful comparative data as to dirt may be obtained by filtering samples through cotton discs, when the discs (which should be supported on wire gauze) are 1 inch in diameter, and a pint of each sample is so filtered. If a vacuum pump is employed, the milk is quickly drawn through the disc.

Eau de javelle completely dissolves such cellular elements as leucocytes, leaving dirt which has gained access since the milk left the udder, so that it may be separately collected. To make this preparation, 20 grammes of good bleaching-powder are rubbed up in a mortar with 100 c.c. of water, and the emulsion mixed with a solution of 20 grammes of anhydrous potassium carbonate dissolved in 100 c.c. of water. After thorough mixing, the gruel-like mass is allowed to stand for an hour, and then filtered under pressure. The clear yellowish liquid keeps well in the dark, but prior to use it should, if necessary, be again filtered.

A. W. F. Lowe has suggested a test for the presence of bile-salts in order to prove that the dirt contains dung. A little grape-sugar is dissolved in a watch-glass containing the sediment, the liquid is then removed as closely as possible by decantation, the sediment is dried at 100° C., allowed to cool, and a drop of pure sulphuric acid is run over the particles, when a fine cherry-red crimson colour appears in the presence of bile-salts. The colour develops around the particles, and it is well to employ a magnifying glass for their examination.

C. Revis has devised the following method for estimating the dirt in milk: A tube holding about 70 c.c. is used. This is made of stout glass drawn out at one end, and a small glass cap is well ground on. Inside the neck of the tube, upon which the cap fits, a glass rod is ground in, of sufficient length to project well beyond the mouth of the tube. The end of the rod is ground flush with the neck, and the cap has such a capacity that when in place $\frac{1}{8}$ inch in depth is left beyond the end of the tube. It is used as follows:

Fifty c.c. of milk (taken with the precaution of thorough mixing) are placed in the tube, the cap being in place, and the rod withdrawn. An india-rubber stopper is put in, and the tube rotated at about 2,000 revolutions per minute for five minutes. (The cap should be well backed up with a pad of cotton-wool to prevent breakage.) The rod is then carefully inserted in the tube, and the cap gently removed with a screwing motion. If stuck, it may

be gently tapped off with a piece of wood (on no account with metal), and the tube thoroughly washed out. The cap is replaced, 50 c.c. of distilled water run in, the sediment stirred up with a platinum needle, and the tube, after well shaking again, rotated for five minutes. The cap and contents are again removed as before, the tube emptied, the cap replaced, and 1 c.c. of eau de javelle (see p. 230) run in on to the sediment. This is mixed up with a platinum needle, and the tube again filled up with 50 c.c. distilled water, shaken, and again rotated. The cap is then finally removed, dried in the water-oven, and weighed. The cap is next cleaned out, dried, and weighed also. The difference equals the amount of dirt by weight in 50 c.c. of milk, after subtracting the weight of a blank eau de javelle experiment—*i.e.*, 1 c.c. of eau de javelle and 50 c.c. of water are placed in a clean tube, mixed, and the cap removed and weighed, giving the weight of residue from the small quantity of dilute eau de javelle solution remaining in the cap on removal. It is, of course, very small. This treatment with eau de javelle completely dissolves leucocytes, etc., mixed with the dirt, but without any action on the dirt constituents. We therefore get a true estimation of the dirt, and the procedure quite excludes any loss during manipulation. The dirt after this treatment may be used for microscopical examination.

It may be well here to refer to the methods by which the leucocyte content may be estimated. The only scientific method is that first suggested by Doane and Buckley, in which 10 c.c. of the milk are rotated, and the number of leucocytes in the deposit, after staining with methylene blue, is found by use of a Thoma-Zeiss blood-counter. The method has been modified by Savage, so that only 1 c.c. of milk is used, and diluted to 20 c.c. before rotation. This is a distinct improvement, as a greater number of cells is usually obtained if the milk be diluted. Probably 1 c.c. is too little; it is better to use 5 c.c. and dilute to 20 c.c. with water, rotate for ten minutes at 2,000 revolutions, break up the cream, rotate again for five minutes, remove all the milk but about 1 c.c., dilute with water to 20 c.c., stir up well, and again rotate. The water is removed to just above the deposit, 4 to 5 drops of saturated aqueous solution of methylene blue added, and the deposit well mixed by blowing through a fine capillary pipette. Stand for fifteen minutes, and carefully dilute with water to 1 c.c. The cells are counted in this dilution and the

usual calculations made. Two concordant counts must be obtained.

" In cows clinically tuberculous the fæces contain large numbers of living tubercle bacilli " (Third Interim Report of the Royal Commission, appointed in 1901, on Human and Animal Tuberculosis), and fæcal contamination is probably responsible for the bulk of the infection of milk by this germ.

The milk must be fresh at the time of analysis, as after lactic acid fermentation of the milk-sugar has set in there is a slight loss in the non-fatty solid matter.

One or two drops of formalin will keep a sample fresh for several days.

The sample should in every case be thoroughly mixed before any part is removed for analysis.

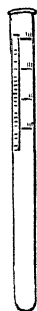



FIG. 32.—THE CREAM TUBE.

The Cream.—Some of the milk is poured into a " cream tube." This is a glass cylinder, the upper part of which bears markings that show the proportion which the cream on separation forms to the total volume of the milk.

The milk is made to stand exactly up to the level of the zero of the scale (due allowance being made for capillarity), and it is then set aside for twenty-four hours. Supposing the cream is found to reach down to 10 on the scale, then it is 10 per cent.; and so the volume of cream is read off against the graduated scale.

Generally the cream will have separated in twelve hours, but the separation is not complete in all samples until twenty-four hours; if the time is protracted beyond twenty-four hours, partial drying ensues, and the resulting contraction will eventually leave a space between the lower surface of the cream and the upper

surface of the milk. Good milk throws about 10 per cent. of cream; but the milk of an Alderney cow may yield between 30 and 40 per cent.

Fresh cream contains a very variable proportion of fat. Generally the amount falls within 40 and 50 per cent.; but there may be 25 per cent. or less when the cream is considerably diluted with milk.

Clotted cream generally contains from 45 to 60 per cent. of fat.

Before **the specific gravity** is taken, any frothing (from shaking or pouring into the glass cylinder) must first be allowed to pass off.

Westphal's balance is a rapid and a more exact method of obtaining the specific gravity of milk than a hydrometer (lactometer) (*vide* p. 8).

The specific gravity of distilled water at 15·5° C. being taken as 1,000, that of pure milk at the same temperature is commonly about 1,032. The fat is so much lighter than the remainder of the milk that with its removal the specific gravity rises even higher still. A specific gravity much above 1,032 will therefore create suspicion as to the removal of the lighter element (cream) from the milk. The addition of water will lower the specific gravity again, for it is obvious that the specific gravity of water being 1,000, the more of this is added the nearer will the specific gravity of the mixture of milk and water be reduced to 1,000. An abundance of cream will also account for a low specific gravity of milk, so that a low specific gravity may mean either abundance of cream or the addition of water.

It follows, then, that the specific gravity and cream tests considered together afford a valuable clue as to the nature of the sample.

The total solids are estimated in the following manner:

Pipette 5 c.c. of milk into a weighed platinum dish. Curdle this by adding to it a few drops of a mixture of one part acetic acid to nine parts of methylated spirit; this will prevent any skin forming on the surface, and will greatly hasten the drying. Dry first on the water-bath, then for two hours inside the water-oven at a temperature not exceeding 105° C. Let cool and weigh. From this total weight subtract the weight of the dish. Multiply the remainder by 20 to bring it to 100 parts. Now convert the 100 c.c. of milk to weight, by deducing this from the specific gravity, and then calculate the percentage of solids by weight.

Example.—Solids + dish weighed 12·903 grammes.
Deduct weight of dish 12·203 ,,
0·700 gramme in 5 c.c.

Multiply by 20 = 14·00 grammes of solids in 100 c.c. of milk.

Now, if the specific gravity of the milk is 1,030, the weight of milk as compared with that of distilled water is as 1,030 is to 1,000; and as 100 c.c. of water weigh 100 grammes, 100 c.c. of the milk will weigh 103·0 grammes. And 14·0 grammes of solids in 103·0 grammes of milk = 13·59 per cent.

Richmond's formula for total solids is: 0·25 G + 1·2 F + 0·14, where F = fat and G = the last two figures of specific gravity and any decimal. The result may be rapidly obtained by means of Richmond's Slide Rule.

Mineral Ash.—Ignite the total solid residue until all dark specks, etc., have disappeared, and nothing but a perfectly clean whitish ash remains. The ignition must be effected slowly and at as low a temperature as possible; Bell recommends that an Argand burner should be used in preference to a Bunsen, on this account.

The ash is then weighed, and its percentage amount by weight ascertained in a similar manner to the total solids. Too large a proportion of ash (that is, above 0·75 per cent.) points to the addition of mineral matter. A milk may have, on the other hand, a paucity of ash, due to the copious admixture of water.

Effervescence on the addition of hydrochloric acid denotes adulteration by a carbonate, which will generally be sodium carbonate. The ash of a pure milk does not effervesce when hydrochloric acid is added to it.

The Fat.—There are many methods in use at the present day for the extraction and estimation of fat. The student is recommended to employ Schmidt's process, and, where necessary or desirable, to corroborate results by Adam's process.

The Werner-Schmidt Process.

This process has become a favourite one, for by it a very accurate estimation of the fat can be made in a short space of time.

The process is as follows:

1. A specially graduated tube, as shown in Fig. 33, is employed to receive 10 c.c. of milk, to which 10 c.c. of strong hydrochloric

acid is added, the milk and acid thus standing to the mark of 20 c.c. on the tube.

2. The mixture is boiled, with frequent shaking, until it turns a brown colour (from the conversion of milk-sugar into maltose and caramel).

3. Let stand for about three minutes, then cool by immersion in a stream of water.

4. Fill up to the 50 c.c. mark with ether; cork the tube and invert it three times; then set aside for fifteen minutes, when the ether will have separated.

5. Accurately pipette off 20 c.c. of the clear supernatant ethereal solution of fat into a weighed flask, and evaporate off

FIG. 33.—STOKES' TUBE FOR THE WERNER-SCHMIDT PROCESS.

the ether, until the last small bubble disappears. A naked flame must not be brought near to the ether, so it becomes necessary to drive off the ether by placing the flask in hot water. The flask can be attached to a condenser and the ether recovered for subsequent use.

6. Dry in air-bath at 100° C., and weigh the residual fat.

7. Next notice how many c.c. of ethereal solution remain in the tube; then from the fat estimated in the 20 c.c. calculate the amount of fat in the whole of the ether.

Example.—Ten c.c. of milk, with a S.G. of 1,031, gives in 20 c.c. ethereal solution 0·277 gramme of fat.

In the tubes there remained 6·5 c.c. of ethereal solution, making a total of 26·5 c.c. $\therefore \frac{26{\cdot}5 \times 0{\cdot}277}{20} = 0{\cdot}367$ gramme of fat in the 10 c.c. of milk, or 3·67 grammes in 100 c.c. But 100 c.c. of milk with a specific gravity of 1,031 weighs 103·1 grammes. Therefore

there are 3·67 grammes of fat in 103·1 of milk, or 3·559 per cent.

Notes upon the Process.—There floats between the brown mixture of HCl and milk and the ethereal solution a fluffy stratum of casein. Three-fourths of this stratum should be taken as ether in reading off the quantity of the latter.

The acid and milk should not be boiled together for more than two minutes, or the ether takes up a caramel-like substance.

The whole process does not take more than forty minutes; and it is well adapted for use where the milk has decomposed.

The boiling with HCl converts the albumin into soluble acid albumin, and the fat is then fully exposed to the action of the ether.

Adam's Process.

This process, by which an extremely thin layer of milk is spread over absorbent paper and the fat then extracted by ether, gives very exact results.

1. Shake the sample, and pipette 5 c.c. into a small beaker about 2 inches deep and 1½ inches wide, then weigh.

2. Soak up as much of the milk as possible—and in every case almost the entire 5 c.c.—by a coil of white demi-blotting-paper, keeping the beaker covered during the absorption.

It is, of course, imperative that the paper should be freed from fat prior to its employment. This may be done by extracting it with acid alcohol (alcohol containing 10 per cent. of acetic acid) for at least three hours, and then thoroughly drying; or the specially prepared slips of fat-free paper made by certain chemical firms may be used. A helical coil is prepared by rolling a strip of the paper upon a glass rod of the size of a cedar pencil, care being taken not to tear the paper; and the coil may be held together with platinum wire.

3. Remove the coil by its upper part, and place it dry end downwards upon a slip of glass, and then re-weigh the beaker with the trace of milk left behind in it. The difference in weight from the previous weighing represents the weight of milk soaked up by the coil.

4. Dry the coil in the water-oven for two hours, and then extract the fat by anhydrous ether in a Soxhlet, twelve siphonings at least being necessary (*vide* p. 9).

5. Receive the fat and ether in a small light flask; drive off

the ether; dry to constancy in a water-oven at about 105° C., the flask being laid in a horizontal position; let cool, and weigh the fat.

Notes upon the Process.—By the addition of ammonia sour milk is as easily taken up as fresh.

A good anhydrous ether may be prepared by placing the commercial article of S.G. 0·720 for three weeks over quicklime, and then distilling.

To obviate the two weighings, some analysts apply the process as follows:

Suspend a strip of fat-free filter-paper over a lighted gas-ring at such a distance above it as would not be too hot for the hand to bear. Distribute over this from a pipette 5 c.c. of milk. When dry, roll up the coil and place it in a Soxhlet's fat-extractor with some anhydrous ether. Cause the ether to siphon over at least twelve times into a weighed flask. Finally, drive off the ether and weigh. Calculate as described above.

For rapid "sampling" purposes the following process is useful, and the results are closely approximate to those obtained by other processes:

M. Gerber's Modification of the Leffmann-Beam Process.

A small test-bottle is employed with a thin, graduated neck; into this is placed 10 c.c. of sulphuric acid (specific gravity, 1·825 at 15·5° C.), 1 c.c. of amyl alcohol (specific gravity, 0·816 at 15·5° C.), and 11 c.c. of the milk sample. The purity and strength of the acid are very important for securing accurate results. The alcohol and milk are allowed to flow down the side of the bottle, so that the three liquids form distinct layers; the bottle is then firmly corked and smartly shaken until the curd is dissolved. It is then held upside down to allow the acid to run down the neck, and this may be repeated two or three times to insure that all the ingredients are well mixed. The bottle is then placed in a centrifuge, which is spun for three minutes (at about 1,000 revolutions per minute); or for nine minutes in the case of separated milk. If only one sample is being tested, another bottle should be filled with milk and placed opposite the sample bottle, in order to balance it in the centrifuge. Upon removal the test-bottle is placed neck downwards into water for two or three minutes at a temperature of 60° to 70° C. to keep the fat liquid. The percentage weight of fat

may now be read off in the graduated neck of the bottle, each of the finer divisions on which indicate 0·1 per cent. by weight of fat. In making this reading the bottle should be held vertically, and by slightly moving the cork at the bottom of the bottle either upward or downward, the lower level of the fat column in the graduated neck may be made to correspond to one of the longer markings which indicate whole percentages of fat, and from this base line the percentage of fat can be easily and rapidly read off.

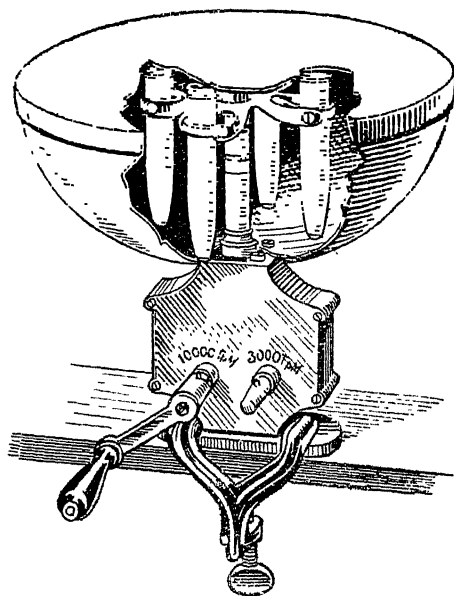



FIG. 34.—CENTRIFUGAL MACHINE, FITTED WITH TWO GEARS, THE LOW GEAR WORKING UP TO 3,000 REVOLUTIONS PER MINUTE, AND THE HIGH GEAR UP TO 10,000.

Hehner and Richmond have devised a formula by means of which the fat in ordinary milk samples may be estimated: $F=0·859\ T-0·2186\ G$, where F=fat, T=total solids, and G=the last two units of the S.G., together with any decimal (*i.e.*, if S.G.=1029·5, G=29·5).

The **solids-not-fat** may be calculated by subtracting the fat from the total solids.

For the estimation of **protein** Kjeldahl's process may be employed (*vide* p. 271). The solid residue of 5 grammes of milk should be worked upon.

Lactose may be determined by Fehling's solution, working with a 5 per cent. dilution of the milk (*vide* pp. 338, 339).

Sour Milk.—Thorpe has shown that the butter-fat suffers little, if any, alteration during the souring of milk, but that the non-fatty solids are more or less affected by fermentative changes. The principal constituent which suffers change is lactose, from a portion of which lactic acid forms at an early stage. As a rule less than half of the lactose (which averages 4·75 per cent. in cow's milk) is thus transformed.

Concurrently with the formation of lactic acid, there are produced products which are either gaseous at ordinary temperatures or are volatilized during the operation of determining the non-fatty contents of the sour milk. The aggregate weight of these substances (acetic and butyric acids, ethyl alcohol, carbonic acid, and traces of ammonia) is not very large, but it is sufficient to affect any estimation of the degree of sophistication to which the milk may have been subjected.

The total loss of solid matter from all causes ranges, as a rule, from only 0·2 to 0·5 per cent. by weight of the milk, and nearly the whole of this is accounted for by the transformation of lactose into alcohol and volatile acid, the changes in the weight of proteid material being relatively insignificant.

Microscopical Examination.—Ten c.c. of milk should be diluted with about 30 c.c. of water, then centrifugalized (at least 1,500 revolutions per minute), the supernatant fluid decanted, the sedi-

FIG. 35.—MILK SHOWING THE LARGE COLOSTRUM CORPUSCLES. (× 250.)

ment washed with water and placed on glass slides, and the contents of the slides examined.

Normal milk under the microscope consists of a collection of round, highly refractile oil globules of about the same dimensions, with an occasional epithelial cell; from three to eight days after calving colostrum corpuscles are also present in larger or smaller quantities (Fig. 35). These latter mostly consist of large yellow cells containing larger and smaller fat globules in their interior.

Where, however, the animal is not in health, the following abnormal constituents may also be found:

Cast of the lacteal tubes, blood-corpuscles (which closely resemble those of the human subject), pus cells and leucocytes, and various micro-organisms (*e.g.*, fungi, such as *Oidium lactis*; moulds, such as penicillium; and bacteria). Blood may be detected either by the spectroscope or microscope. When present in considerable quantities, blood tinges the milk and has a tendency to settle as a brown deposit; or after warming the milk to about 50° C., a high-speed centrifuge may furnish a red deposit in the milk tube.

Cow-dung shows vegetable parenchyma and vessels of a distinct yellow tint, with a great deal of brown débris.

In the deposit there may be also detected yeast cells, cotton fibres, hairs, starch granules, sand particles, etc.

All milk samples contain a certain number of cellular elements, and in certain pathological conditions their number is enormously increased. Deductions of great value can be made from accurate determinations of their number in the milk of individual cows, but for mixed milk samples the determination is of much less value. If the diluted milk is centrifugalized, the sediment washed (preferably by heated ox serum, as recommended by Hewlett and Revis), the sediment spread evenly over a cover-slip, dried, treated with ether to remove fat, and stained by methylene blue, the number of leucocytes may be enumerated (*vide* p. 231).

It has been suggested that the number of these cells should not exceed 500,000 per c.c., but it is probable that such a limit would at times lead to the condemnation of milk derived from healthy sources. These cellular elements have been usually regarded as leucocytes or pus cells, and hence it is that a standard has been suggested for the hygienic control of milk. There are good grounds, however, for believing that most of the cells found are young epithelial cells and not leucocytes. Savage has classified these cellular elements into polymorphonuclear cells, lymphocytes, large leucocytes, and doubtful cells.

Owing to the difficulty of recognizing pus cells from other cells that may be present, it is not easy to say how frequently pus resulting from inflammatory processes in the udder gets into milk; but in a large percentage of cases cells indistinguishable from pus cells are present. Lymphocytes, which stain deeply and possess

nuclei which occupy nearly the whole of the cells, are not normal constituents of milk except within three weeks of parturition; but, like polymorphonuclear leucocytes, they are occasionally present for a few days in the milk of an apparently healthy cow. These bodies are found associated with staphylococci, diplococci, and streptococci generally in diseases of the udder, and they are to be found associated with tubercle bacilli in tubercular mastitis. A high cell count accompanied by streptococci apparently indicates some udder trouble.

CHAPTER III

THE SOPHISTICATION OF MILK—MILK PREPARATIONS—MILK STANDARDS—BACTERIOLOGICAL NOTE

Cow's milk should be the normal, clean, and fresh secretion obtained by completely milking the udder of the healthy cow, properly fed and kept.

About 10 per cent. of all the samples of milk taken under the Sale of Food and Drugs Acts, in England and Wales, are adulterated.

The Addition of Water.—Whether milk is naturally poor or has been made so by the addition of water, the dairyman who sells it defrauds the purchaser, for the latter demands and pays for pure milk of average quality.

It is clear that the percentage amount of both fatty and non-fatty solids will be reduced by any addition of water; but the estimation of the amount of added water is always made from the *non-fatty solids*, because these depart less from the average than is the case with the fatty solids. The legal low limit for non-fatty solids is one of 8·5 per cent.

Supposing, then, a sample yields 8 per cent. of non-fatty solids. Then if 8·5 per cent. of non-fatty solids denotes 100 per cent. of pure milk, 8 per cent. denotes only about 94 per cent. of pure milk.

Therefore there is about 94 per cent. of *pure* milk in the sample, and ($100-94=$) 6 per cent. of water has been added.

The ash should in every case be low when the solids-non-fat are low, or some mineral adulterant has been added.

Cream Abstraction.—Though the milk from the same cow may vary at times, the mixed product of many animals ("dairy samples") varies but little.

The legal low limit of 3 per cent. of fat is one which is reached

by all genuine dairy samples obtained from a fairly good herd of cows, kept and fed under average conditions; though, as Bell and others have shown, the milk of individual cows may sometimes fall below this limit.

The percentage reduction of fat (by the removal of cream) is a simple calculation after an estimation of the fat has been made. Suppose that the fat has been found to amount to just 2·5 per cent. Then 3 per cent. – 2·5 per cent. = 0·5 per cent. of deficiency in fat; or $\frac{2 \cdot 5 \times 100}{3} = 83 \cdot 3$ per cent. of the original fat remains and (100 – 83·3 =) 16·7 per cent. of the total fat content of the milk has been removed. In cases where the fat is low and the solids-non-fat are high there can be little doubt that fat has been abstracted, and that the low fat is not due to the dilution of the milk with water.

The "toning down" of good milk by the addition of separated milk is much practised, and large numbers of the samples analyzed are found to barely reach the low legal limit of 3 per cent. of fat.

As milk stands, a certain proportion of the fat quickly rises to the upper layers, and a defence is sometimes set up by the dairyman that a poor sample was due to the fact that such top milk had all been sold, and the sample was some of the last of the milk in the can. This defence is, in many cases, a well-recognized subterfuge, for it is the duty of the vendor to mix the milk and to supply fair samples to one and all alike. Failure to draw off the "strippings" no doubt often accounts for the low figure of fat in milk.

Samples collected on Sunday mornings are generally amongst the poorest, for dishonest tradesmen adulterate on these days in order to meet the extra demand, due to the fact that more people take their meals at home on that day.

In addition to water, there are *other adulterants* added to milk. Chalk and starch were formerly used, but they are very rarely, if ever, employed at the present day. Sodium carbonate is rarely used to preserve the milk and to neutralize it when sour. It may be tested for (E. Schmidt) by adding 10 c.c. of alcohol to 10 c.c. of milk, followed by a few drops of 1 per cent. solution of rosolic acid. Pure milk yields a brownish-yellow colour, but if sodium carbonate or borax is present a more or less marked rose-red colour appears.

Boric and salicylic acids, borax, "formalin," benzoates, fluorides, peroxide of hydrogen, have been used as milk preservatives. Either a mixture of boric acid and borax, or "formalin," was most generally employed. Such chemical preservatives are now prohibited in any form of milk by the Public Health (Milk and Cream) Regulations, 1912.

Boric acid with borax was largely added to milk during the summer months, and the amount generally employed was about 5 grains to the pint. Experiments go to show that not less than 4 grains of a mixture of boric acid and borax are necessary to preserve a pint of milk for twenty-four hours in warm weather (Rideal, Foulerton). Salicylic acid was not so frequently employed, because of its lesser solubility and unpleasant taste.

"Formalin" is a commercial preparation containing about 38 per cent. of formaldehyde.

"Mystin" (a mixture of formic aldehyde and sodium nitrite) has occasionally been employed as a preservative.

Annatto and turmeric, coal-tar dyes and saffron, are yellow colouring agents which are added to give the milk a rich yellow appearance. Annatto and coal-tar dyes are chiefly employed. During the Great War an Order (No. 1,317) of the Ministry of Food required, *inter alia*, that no colouring matter was to be added to milk not sold for consumption on the premises of the seller.

The tests for the antiseptic and colouring agents in milk are given in Chapter XIII., which treats of the subject of Antiseptics and Colouring Agents in Food.

Cream.—Starch or gelatine is sometimes added to cream to thicken it. Gelatine may be detected by adding to 10 c.c. of the sample 20 c.c. of cold water and 10 c.c. of a solution of acid nitrate of mercury. The whole is then well shaken, allowed to stand for five minutes, and then filtered. If much gelatine is present, a clear filtrate cannot be obtained. A portion of the filtrate is mixed with an equal quantity of a saturated aqueous solution of picric acid, when a yellow precipitate forms if gelatine is present (Stokes). Starch is detected by the blueing with iodine solution.

Milk solids and other fats, and lime in cane-sugar syrup, have also been added to cream. Sucrate of lime may be detected by estimating the lime in the ash of the cream (average percentage

of CaO=22 per cent. of the ash). The same preservatives are employed as in the case of milk.

Under the Public Health (Milk and Cream) Regulations, 1912, no preservative may be added to cream which contains less than 35 per cent. by weight of milk fat, whereas in cream containing 35 per cent. or more of milk fat the only chemical preservatives permitted are boric acid, borax, or a mixture of these, and hydrogen peroxide; but the addition of these preservatives is subject to a system of declaration. Furthermore, no thickening substance may be added to cream. In these Regulations "thickening substance" means sucrate of lime, gelatine, starch paste, or any other substance which, when added to cream, is capable of increasing its thickness. Neither cane nor beet sugar is to be regarded as a preservative or as a thickening substance. By the Public Health (Milk and Cream) Regulations, 1912, Amendment Order, 1917, it is required that boric acid or borax must not exceed 0·4 per cent. (28 grains per pound) by weight of the preserved cream, and that declaratory labels must be affixed to any receptacle containing it.

Hand-skimmed milk is sometimes made to look like good rich milk by the addition of *condensed milk*. An analysis of the ash and non-fatty solids will detect the fraud, since these will both be in excess of their general proportions (more especially the sugar), and the amount of soluble albumin will be diminished (Faber).

Hand-skimmed milk is generally slightly acid, and the specific gravity is about 1032·5. The fat generally amounts to from 0·5 to 1·5 per cent.

The bulk of the samples of "**separated milk**" contain from 0·2 to 0·3 per cent. of fat.

Skimmed and separated milk must legally contain at least 8·7 per cent. of solids-non-fat.

Separated milk is sometimes "enriched"—that is, the butter-fat taken out by the separator is replaced by an emulsion of some other fat. In such case a separate analysis of the fat of "the cream" must be made by the Reichert-Wollny process, as described in the analysis of butter.

Condensed milk may be unsweetened; but more generally it is sweetened—whole milk being concentrated to about one-third of its original volume, and cane-sugar added; or it may be prepared from sweetened skimmed or separated milk.

The following table, taken from Dr. Coutts's Report to the Local Government Board (1911), indicates the percentage composition of the chief classes of condensed milk upon the market:

	Full Cream.				Machine Skimmed.	
	Sweetened.		Unsweetened.		Sweetened.	
	Lowest.	Highest.	Lowest.	Highest.	Lowest.	Highest.
Total solids ..	68·1	83·6	29·2	38·0	56·9	79·1
Protein ..	7·3	11·4	8·0	10·0	7·6	12·3
Fat	8·0	13·7	8·2	11·9	0·1	6·5*
Lactose ..	11·6	17·6	11·1	16·0	10·9	17·0
Ash	1·6	3·4	1·6	2·5	1·6	2·9
Cane-sugar ..	36·1	44·6	Nil	Nil	30·4	52·6

In the analysis of condensed milk 20 grammes should be taken and made up to 100 c.c. with water as a stock solution.

For Total Solids.—Evaporate 5 c.c. of this as in the case of milk.

For Ash.—Incinerate the above. The ash averages 1·9 to 2 per cent.

For Fat.—Estimate by Adam's process. The fat averages about 10 per cent.

The best method for obtaining a rapid and accurate estimate of the fat in sweetened brands is the Gottlieb process (the Werner-Schmidt process being inapplicable):

Into a graduated 100 c.c. tube put 10 c.c. of above solution; add 1 c.c. of 40 per cent. ammonia; warm to 30° C.; shake; add 10 c.c. alcohol (95 per cent.), and shake. Add 25 c.c. ether, and shake; add 25 c.c. petrol-ether, and shake; let settle; pipette off 25 c.c. of the mixed ether solution, evaporate this and weigh. Calculate as in the Schmidt process. The fat of sour milk, cream, cheese, butter, etc., may all be reliably estimated by this process.

For Total Sugars (cane and milk).—To 10 c.c. of stock solution add 40 c.c. of methylated spirit, add one drop of acetic acid, shake (this will precipitate the curd and fat), filter. Evaporate 20 c.c. of the filtrate, and weigh the residue. Now incinerate this, and subtract this ash from the total sugar weight. Multiply the difference by 22·5, and then by 5, to give percentage of mixed sugars. Apparently one should first multiply by 25, but allow-

* A partly skimmed milk.

ance has to be made for the volume occupied by the precipitated curd and fat. Deduct milk-sugar (estimated by Fehling's solution), and the difference is cane-sugar.

The milk-sugar (which averages 13 to 15 per cent.) is determined by titrating a 5 per cent. dilution of the milk with Fehling's solution (*vide* pp. 338, 339). The cane-sugar may subsequently be estimated by boiling the stock solution with citric acid, which inverts the cane-sugar; the solution is cooled, neutralized with potassium hydroxide solution, made up to a known volume, and titrated with Fehling's solution.

Many brands of condensed milk contain a very large amount of sugar, the average being 38 to 40 per cent.; while others are unsweetened. When opened, the latter have inferior keeping powers.

For Proteids.—Perform Kjeldahl's process on 10 c.c. of stock, and multiply the N by 6·38. The proteid matter averages 9 per cent.

The " degree of condensation " may be approximately gauged by dividing the percentage of solids by 12·6, when the condensed milk is unsweetened; or by dividing the percentage of fat by 3·6 in other cases—12·6 per cent. and 3·6 per cent. being, respectively, the average amounts of total solids and fat in milk.

Brands of condensed whole milk (not " machine skimmed ") ought to contain at least 10 per cent. of milk-fat and 25·5 per cent. of non-fatty milk solids, of which the ash should constitute about 2 per cent.

Heated Milk.—Sometimes it is required to know whether milk has been sterilized or boiled. In such a case 3 c.c. of milk may be mixed with 1 c.c. of a freshly prepared 10 per cent. solution of hydroquinone, and about 15 drops of hydrogen peroxide added. If the milk has not been raised to a high temperature, an immediate rose colour forms, but otherwise no colour is produced, as the enzyme responsible for the reaction has been destroyed.

Another test (Storch) consists in adding a drop of hydrogen peroxide solution and 2 drops of a 2 per cent. solution of paraphenylene diamine to 5 c.c. of milk in a test-tube, and shaking. The milk becomes an indigo-violet colour if it has not been heated above 78° C.; otherwise the colour remains white.

The following changes result when milk is boiled: Carbonic acid gas is expelled, and the calcium and magnesium salts are therefore partially precipitated; the greater part of the phosphates are also precipitated. There is a slight diminution in

the organic phosphorus originally present, a partial decomposition of the proteins. The skin which forms on the surface (solely when the heating is done in an open vessel) consists mainly of lactalbumin. This pellicle has approximately the following composition: Fat, 45·5 per cent.; lactalbumin and casein, 51 per cent.; mineral ash, 3·5 per cent. The normal emulsion of the fat globules is disturbed, so that the cream does not rise to form a layer on the surface, the lactose is partially burnt ("caramelization"), and the milk therefore becomes slightly brownish in colour, and its flavour is somewhat altered. The boiling destroys the ferments in the milk, and probably also the antiscorbutic element of raw milk; the natural germicidal power of fresh raw milk is lost, and almost all the bacteria are destroyed, those left consisting of sporing forms and certain highly resistant varieties.

None of these changes take place, appreciably, in "low temperature pasteurization"—namely, the heating of milk to a temperature of 60° C. for thirty minutes—except the great reduction in micro-organisms.

Milk Powders.—These are now very largely made, both from whole milk and from skimmed milk. The powders are cream-coloured, with a slight distinctive odour. The fat in whole-milk powder should amount to at least 25 per cent.; the protein to at least 24 per cent.; and the lactose 35 per cent. The moisture should not exceed 5 per cent. The fat can be determined by the Gottlieb process or by the Soxhlet method. Otherwise the analysis follows on the general lines already described.

"**Koumiss**" consists of milk which has been skimmed of some of its cream and sugar added; it is then partially fermented by yeast or other ferments, whereby much of the sugar is converted into lactic and carbonic acids.

Bean or Synthetic Milk.—In the preparation of this milk soya beans are washed and soaked in water, the outer integuments being removed. The softened beans are then ground between millstones and the powder boiled with water and filtered through fine sieves. A cream-coloured liquid results, closely resembling milk, but with a distinct beany odour and taste. For use, sugar is added to suit the taste of the consumer. Bean milk on analysis contains about 2·1 per cent. of fat, 3·7 per cent. protein, 1·4 per cent. carbohydrates other than sugar, and 0·4 per cent. of mineral ash.

Milk Standards.

In addition to the legal standards at present in force, certain other standards are advocated. Only a small proportion (one-sixth to one-eighth) by weight of the cow-dung which finds its way into milk is recoverable (as dirt) from milk by centrifugalization; but despite the difficulties involved, certain standards for dirt in milk have been suggested. Most authorities agree that milk yielding more than 1 part of recoverable dirt per 100,000 is dirty. Houston suggests that, as a working standard, (1) the deposit from a litre of milk obtained by sedimentation in a special cylindrical separating funnel after twenty-four hours should not exceed 1 part per 10,000 by volume; and (2) when the deposit from (1) is centrifugalized, it should not exceed half the above amount.

It has been suggested that, as a general rule, the recoverable dirt should not be allowed to exceed 2 parts per 100,000 by weight, and some advocate a standard as low as 1 part. As a rough household standard, ½ pint of milk placed in an ordinary tumbler should not throw a visible sediment in two hours. But dirt may be partially removed by trade filtration, which leaves behind the harmful bacteria, and therefore the only satisfactory standards are those based upon bacterial counts.

Seasonal standards of total bacterial counts are serviceable. In Chicago, for instance, 1,000,000 bacteria on gelatine at 20° C. per c.c. of milk from May 1 to September 30, and half that amount for the remainder of the year, is the standard of milk as it arrives in that city. Savage prefers a standard of lactose fermenters of the coli type of not more than 100 in winter and 1,000 in summer, and he suggests that initial contamination may be best judged from the number of *Bacillus enteritidis sporogenes* (the spores of which abound in cow-dung), as that organism shows relatively little tendency to multiply in milk.

Certainly leucocytes exceeding 1,000 per c.c. along with many streptococci suggests the desirability of investigation.

In special (certificated) milk, which is sold at an enhanced price, it is possible to impose such high standards as—freedom from *B. tuberculosis*, a total bacterial count below 10,000 per c.c. (Class A) and 100,000 per c.c. (Class B), and delivery to the consumer at a temperature of not above 10° C.

A suggested standard for pasteurized milk is that the total

bacterial count should not exceed 1,000,000 per c.c. prior to pasteurization, and 50,000 per c.c. when pasteurized and delivered to the consumer.

A reductase test (Schmidt-Muller) may serve as a standard for freshness. The test reagent is made by adding 5 c.c. of a saturated alcoholic solution of methylene blue (zinc chloride double salt) to 195 c.c. of distilled water; it should be boiled every day before using. One c.c. of the reagent is mixed with 20 c.c. of milk, the surface is sealed with paraffin, and then the test-tube and its contents are placed in a water-bath at 45° C. to 50° C. Fresh milk should remain blue for twelve hours or more. The reduction of methylene blue by raw milk (in the absence of formalin) is due to bacterial contamination, and if the milk decolorizes within one hour the organisms certainly exceed 500,000 per c.c.

Bacteriological Note.

Milk as secreted is free from organisms, but even in the milk cistern of the udder and in the teat canals some bacterial infection takes place; while at every stage, from the udder to the consumer, contamination with bacteria is possible, and under many of the conditions which now prevail is invited. Organisms gaining access to milk, unlike those in air and water, are usually in an environment most favourable to multiplication, and as a consequence milk as vended frequently contains one to five millions or more organisms per c.c., the number, as is to be expected, being considerably greater in hot weather.

Park* has shown that in New York, "with only moderate cleanliness, such as can be employed by any farmer without adding appreciably to his expense—namely, clean pails, straining-cloths, cans or bottles, and hands, a fairly clean place for milking, and a decent condition of the cow's udder and the adjacent belly—milk when first drawn will not average in hot weather over 30,000 and in cold weather not over 25,000 bacteria per c.c. Such milk, if cooled to and kept at 50° F., will not contain at the end of twenty-four hours over 100,000 bacteria per c.c. If kept at 40° F. the number of bacteria will not be over 100,000 after forty-eight hours."

The estimation of the number of bacteria in milk, or of some special group of bacteria such as the *B. coli* group, is the natural

* *Journal of Hygiene*, 1901, vol. i., p. 391.

measure of the degree of contamination of milk, but the question of the numbers to allow is beset with difficulties, largely owing to the suitability of milk as a medium for the propagation of bacteria.

A milk initially comparatively pure will frequently show after the lapse of twelve to twenty hours many more bacteria than one collected under much less cleanly conditions, and initially much more heavily charged with bacteria, but examined after the lapse of only three or four hours from milking.

These differences in the bacterial content are not only determined by the initial contamination and by the time since milking, but also by the temperature at which the milk has been kept, and the latter introduces a condition subject to great variation.

The milk, after careful mixing, should be collected in sterile glass-stoppered bottles. Those used for the bacteriological

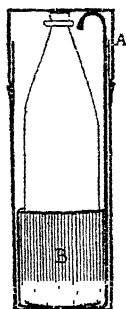



FIG. 36.—DÉLÉPINE'S MILK-COLLECTING APPARATUS.

examination of water may be used, or the simple and efficient apparatus described by Délépine may be employed.

It consists of a metal case containing a 7 or 8 ounce bottle and a milk-scoop. All the parts are thoroughly sterilized in the laboratory before being sent out, and the sterilized case is opened only at the time when the sample is taken. The sterilized scoop is used to remove the milk from the cans or other vessels. When obtained direct from a suspected cow, the milk may be milked into the scoop. The metal cases are packed in refrigerating boxes if necessary.

If the sample cannot be examined within an hour or so, it must be transmitted packed in ice.

If milk from individual cows is being collected, the teats and the milkers' hands should be washed and disinfected. In some cases it is necessary to collect a separate sample from each quarter, while for a complete examination fore, middle, and end milk samples should each be collected.

As a rule condensed milks are free from preservatives. In the sweetened milks the sugar is sufficient to inhibit the growth of bacteria, and in the unsweetened the milk has been sterilized at temperatures over 100° C. The processes carried out in condensing the milk are sufficient to destroy *Bacillus coli, B. tuberculosis,* and other pathogenic organisms, but spore-bearing bacilli, streptococci, sarcinæ, yeasts, and other saprophytes, are often present, so that condensed milks must not be regarded as necessarily sterile. It is probable that the bulk of the organisms present have gained admission during the processes of cooling and of filling the tins.

At the temperature of about 10° C. the multiplication of lactic acid forming bacteria is checked, and those organisms are destroyed at a temperature of 70° C. maintained for twenty minutes. The ferments hitherto detected in cow's milk (peroxidase, reductase, catalase, etc.) are mainly derived from bacteria; but certain of such ferments which are found to be present in uncontaminated milk, such as amylase, do not appear to be of value in digestion and nutrition. The chief enzymes are destroyed at about 70° C. in thirty minutes. At 80° C. all enzymes are destroyed; but at 60° C. their activity is, if anything, slightly promoted.

The discovery by Ehrlich that passive immunity could be produced by suckling when the mother was immune led to the investigation of the presence in milk of precipitins, agglutinins, opsonins, antitoxins, and other so-called "protective substances" in milk. These are all destroyed at about 60° C.

There are also present in milk certain biological bodies (hormones and vitamines) produced by the direct action of living cells. As it appears that these biological bodies are not absorbed in the alimentary canal, it is not likely that they act as antigens in the infant. The vitamine present is not destroyed at the temperature of boiling milk.

CHAPTER IV

BUTTER—CHEESE—LARD

BUTTER.

AN average sample of fresh butter has the following **composition:**

Fat, 83·5 per cent.
Curd (casein), 1 per cent.
Ash, 1·5 per cent.
Milk-sugar, 1 per cent.
Water, 13 per cent.

The water may vary from 8 to 15 per cent.

The butter-fat is a combination of glycerol with certain fatty acids; and consists of—

(*a*) The glycerides of certain volatile fatty acids, *soluble* in hot water—*i.e.*, principally butyric, but also smaller quantities of caproic, capric, and caprylic acids.

(*b*) The glycerides of certain fatty acids, *insoluble* in hot water —*i.e.*, palmitic, stearic, oleic, and myristic acids.

The glycerides contain several acid radicles in the same molecule.

It is desirable to proceed with the analysis of butter without delay, and if it has to be kept it should be stored in a cool, dark place pending analysis; for so soon as butter commences to undergo decomposition, some of the characteristics which distinguish true butter-fat from other fats become less marked. On becoming rancid, the insoluble fatty acids tend to increase, and the soluble fatty acids to diminish. Rancidity of butter-fat is brought about by micro-organisms in the presence of light and air, and it spreads from the outside inwards. It sets in early when the butter-milk is not properly washed out.

The portion to be analyzed should not be wrapped in paper

(which will absorb moisture), but should be placed in a clean dry bottle, carefully sealed.

Physical Characters.—The odour and taste of good butter are familiar to everyone, and are so characteristic that they form in themselves useful evidence of its purity. If butter is heated to 21° C., any unusual taste becomes more appreciable.

With regard to the colour, the same remarks made in connection with the colour of milk apply to the butter made from it.

The **water, fat, curd, and salt in butter** may be estimated as follows:

Weigh 5 grammes of butter into a weighed flat-bottomed dish, and place for about three hours in a drying-oven at 105° C.; then cool and reweigh, and the loss represents water. Or 2·5 grammes of butter may be placed in a tared flat-bottomed beaker, and put into the hot-air oven for several hours at a temperature not exceeding 105° C.—until no more globules of water can be seen on looking at the glass beaker from below, and until a constant weight is obtained. The fat may be estimated by extracting the water-free butter with ether. The washing should be repeated several times with fresh ether (which is decanted cautiously after each washing); the residue is then dried and weighed, and thus the weight of curd and salt is obtained, and from the loss in weight the fat is estimated. The salts may be weighed after incinerating the fat-free residue at as low a temperature as possible.

Adulteration.—All foreign fats made up to resemble butter, and whether mixed with butter or not, have now to be labelled and sold as "margarine."

The Butter and Margarine Act, 1907, requires that a limit of 16 per cent. of water shall not be exceeded in butter and margarine, with the exception of "milk-blended" butter, which may contain 24 per cent.; but the latter may only be sold by a name which is approved by the Board of Agriculture as not suggestive of butter.

The Sale of Food and Drugs Act, 1899, makes it unlawful to manufacture, sell, expose for sale, or import, any margarine the fat of which contains more than 10 per cent. of butter-fat.

In the manufacture of **margarine** animal and vegetable fats are melted, strained, cooled with ice, worked up with a little milk, artificially coloured and salted; the result is an article very similar in appearance and taste to ordinary butter, and but little

inferior, if at all, in nutritive qualities. It constitutes a good article of food, but it must be labelled and not sold as butter. Lard, beef and mutton fats, together with vegetable oils (cotton-seed, sesame, cocoanut, earth-nut), have been employed as substitutes of butter-fat. Paraffin or petroleum oil and solid paraffin-wax have rarely been incorporated with margarine. Paraffin has no food value, and it is liable to prove deleterious to health. Some control is needed over the oils employed in the manufacture of margarine.

The lines upon which to proceed in order to detect whether the sample consists of pure butter-fat, an admixture of this with other fats, or of these prepared foreign fats alone, must be those which take advantage of the differences existing in the composition of the fats.

The fat of the butter or margarine may be separated and collected in the manner described on pp. 6, 7.

The following are important differences:

Butter-Fat.	The other Fats most used as Substitutes.
1. The soluble volatile fatty acids form between 6 and 7 per cent. on an average, and are never below 4·5.	1 Constitute rarely more than ½ per cent , and never more than ¾ per cent.
The insoluble fatty acids form about 88 per cent	Generally about 95 per cent
2 The Reichert-Wollny figure (5 grammes) is from about 24 to 32.*	2 The figure is generally from 1 to 2, except in respect of cocoa-nut oil, when it is from 7 to 8.
3. By the Valenta test the fat clears at 30° to 40° C.	3. No animal fats clear below 94° C., and no vegetable oil used as a substitute clears below 80° C.
4 *Polariscope.* — When a thin layer of the sample is examined by the micro-polariscope, at the moment the Nicol prisms are crossed, the whole field is dark, except for a few minute specks due to preservatives, etc.	4. Similarly treated, any mixture containing more than 40 per cent. of foreign fat shows a field full of light, cloud-like forms. In fact, it is impossible to get a dark field Since mixtures of butter and margarine are rare, this is the best physical test. It really shows that the fat used has been melted.

By far the best evidence of the presence of foreign fat is derivable from the amount of soluble and volatile fatty acids present; and this is best obtained by the following method:

The Reichert-Wollny Process.—The volatile and soluble fats have been seen to be relatively much higher in butter-fat than in

* Some pure Irish butters have furnished figures of between 20 and 24

the other fats used as substitutes. If, then, the volatile fatty acid is separated, the acidity which it furnishes in the case of butter-fat will be considerably greater than any acidity furnished by other fats similarly treated.

1. The recently melted butter-fat, filtered through a dry filter, is poured into a weighed small-necked glass flask (200 c.c. capacity) until the scales register the addition of 5 grammes of butter-fat at about 38° C. If fat in slight excess of this weight is added, the excess may be removed by a glass rod, and with a little care it is not difficult to weigh out precisely 5 grammes.

2. Two c.c. of a 50 per cent. soda solution, and 10 c.c. of about 92 per cent. alcohol, are then added to the flask, which is fitted with a vertical glass tube to act as a reflux condenser;

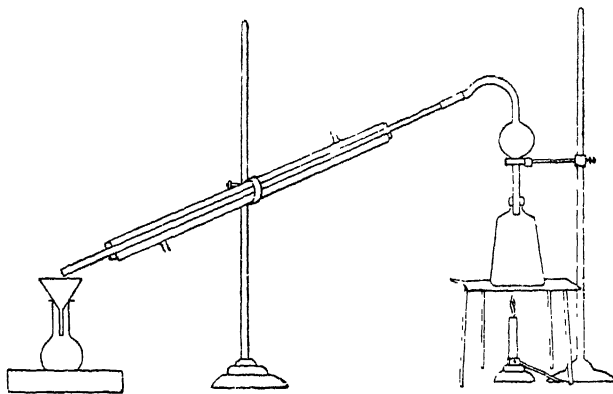



FIG. 37.—APPARATUS FOR THE REICHERT-WOLLNY PROCESS.

it is then placed on a water-bath and heated for a quarter of an hour, the flask being gently rotated from time to time. Soaps are thus formed by the combination of the fatty acids with the alkali.

The cork and tube are now removed, and the alcohol distilled off by heating the flask for about half an hour, or until the soap is dry. Any traces of alcohol remaining in the flask can be removed by sucking the air out through a narrow tube passed through a cork inserted in the flask.

3. One hundred c.c. of hot distilled water, which has been kept boiling for ten minutes, are next added, and the whole gently heated, with occasional shakings, until the soap is completely dissolved.

4. Forty c.c. of dilute sulphuric acid (1 in 40) are poured in after the soap solution has cooled to about 62° C.; the soap is thus decomposed, and the fatty acids are set free.

The flask is restoppered, when the fatty acid emulsion is fused by a gentle heat, and then allowed to cool.

5. Two pieces of pumice of the size of a pea are added to prevent bumping, and the flask is then connected to a small condensing apparatus by means of a glass tube 7 millimetres wide and having at a distance of 5 centimetres above the cork a bulb of a diameter of 5 centimetres; the tube is bent immediately over the bulb upwards in an oblique angle, in which direction it extends for 5 centimetres, and is then again bent downwards, also obliquely. Connection is made with a condenser by means of an india-rubber tube, and the contents of the flask are gradually heated and made to boil slowly. The insoluble fatty acids are melted and the butyric acid is distilled over unchanged, but the distillate also contains some of the insoluble volatile fatty acids, and these must be separated by allowing the distillate to run through a dry filter before it is finally collected.

6. Exactly 110 c.c. of the filtered distillate are collected in a graduated flask (the flame being regulated in such a way that the distillation lasts thirty minutes), and the acidity of the distillate is estimated by means of a decinormal solution of an alkali (baryta best), phenolphthalein being used as indicator. To the figure thus obtained the amount found by a blank experiment with the alcoholic soda solution (made at the same time and under precisely similar conditions) is subtracted. This deduction ought not to amount to more than 0·3 c.c. of the $\frac{N}{10}$ alkali. The result is known as the "Reichert-Wollny number."

Five grammes of pure butter-fat treated in this manner do not yield a less amount of acidity than corresponds to a Reichert-Wollny number of 24* (24 c.c. of decinormal soda or baryta solution), and the "number" may be from 24 to 32. Oleomargarine furnishes a number generally from 1 to 2, as a little milk is usually churned in to give a butter flavour, and this may account for as much as 5 to 6 per cent. of butter-fat. In this country, in order to make the practice of fraud more diffi-

* Some pure Irish butters have, however, furnished figures of between 20 and 24.

cult and its chemical detection more easy, more than 10 per cent. of butter-fat in margarines is prohibited by the Sale of Food and Drugs Act, 1899. This amount of butter-fat in margarine will furnish a Reichert-Wollny number of 4.

The Society of Public Analysts has decided that the amount of butter-fat in margarine, when it exceeds the legal limit of 10 per cent., shall be assumed to be as follows:

Reichert-Wollny Number of the Mixture.	Percentage of Butter-Fat present in the Mixture.
4·0	10
4·3	11
4·6	12
4·9	13
5·2	14
5·5	15
5·9	16
6·2	17
6·5	18
6·8	19
7·1	20

Supposing the Reichert-Wollny figure is 20 c.c., what would be the percentage of pure butter-fat in the sample? Taking 2 as the highest possible figure for other fats (in the absence of cocoa-nut oil), and 24 as the lowest figure for butter-fat, a difference of 22 (24−2) would represent 100 per cent. of genuine butter. The per cent. of genuine butter in a mixture with a figure of 20 would be represented by (20−2) 18. Now if 22=100 per cent. of genuine butter, 18—82 per cent.; and 100—82=18 per cent. of the total fat would be foreign.

Note.—By means of a T-piece in the tube by which the flask is connected with the condenser, the alcohol can be distilled off and water added to the residual soap without opening the flask and exposing the contents to the CO_2 of the air.

Leffmann and Beam substitute a solution of sodium hydrate in glycerol for the alcohol and sodic hydrate used as the saponifying agent; this dispenses with the use of alcohol, and so prevents the results being vitiated by the absorption of CO_2 during evaporation, and it shortens the time required for the process. The solution is made by allowing a 50 per cent. solution of NaHO to stand twenty-four hours to clear, then well mixing 20 c.c. with 180 c.c. of pure glycerine.

Polenske has modified the process in a way which increases its value for the purpose of detecting the presence of cocoanut oil in butter:

1. When the 110 c.c. of distillate have been collected, the receiver is replaced by a 25 c.c. cylinder.

2. Without mixing its contents, the receiver is now placed in a bath of water at 10° C.—the water surface of which comes to the level of the 110 c.c. mark, or just above it.

3. The insoluble fatty acids rise to the surface in the receiver; and in the case of butter they form a solid mass of white opaque granules, whilst with pure cocoanut oil only oily drops are obtained. Mixtures containing more than 10 per cent. of cocoanut oil also yield oily droplets.

4. After mixing and filtering the contents of the receiver, the Reichert value is determined on the filtrate.

5. The condenser-cylinder and receiver are washed with 18 c.c. of water, which are then poured over the filter.

6. The insoluble fatty acids on the filter are now dissolved in alcohol, and the solution titrated with $Ba(OH)_2$ solution, using phenolphthalein as indicator.

7. The number of c.c. of $\frac{N}{10}$ barium hydroxide solution required is termed the "new butter value" of the fat under examination. Genuine butter does not give "new butter values" exceeding 3·0.

The addition of cocoanut oil raises this figure, as cocoanut oil with Reichert figures between 6·8 and 7·7 gives "new butter values" from 16·8 to 17·8.

If 3 c.c. of the melted fat is mixed with 3 c.c. of glacial acetic acid (S.G. 1056·2) in a narrow graduated tube and a thermometer inserted, it will be seen that margarine does not form a clear solution when the mixture is warmed and well shaken up until a temperature of 94° C. is reached, but butter generally clears at about 36° C. (**Valenta test**). Genuine butter samples vary some what as to the temperature at which they clear, and the variation falls between 30° and 40° C.; but no animal fats clear below 94° C., and no vegetable oil of common use clears below 80° C. The test may be applied by discontinuing the heat after complete solution has taken place, retaining the thermometer in the solution, and taking the temperature at which the liquid becomes turbid. The clarifying temperature is taken as half-way between that noted when the mixture first cleared and that when the mixture commenced to become turbid again.

It is important to exclude moisture from the material to the tubes employed, as this raises the Valenta figure. As the strength of the acetic acid used determines the figure obtained, it is as well to check results by obtaining the figure upon a sample of butter-fat of undoubted purity.

Jean has extended the test as follows: After a few minutes it is seen how much acetic acid has separated out, since some of the acid is absorbed by the fat. Suppose the level of the acetic acid after the experiment was 1·1 c.c., then the acetic acid absorbed by the butter$=3-1{\cdot}1=1{\cdot}9$ c.c., and 1·9 c.c. in 3 c.c.= 63 per cent. Butter-fat absorbs over 60 per cent., while the fat of margarine rarely absorbs over 30 per cent.

A useful rough test for margarine is to add 5 grammes of the fat to 50 c.c. of fresh milk, which has been heated almost to the boiling-point, and stir the mixture with a wooden stick until the fat is melted. Then place the beaker in ice-cold water, and continue stirring until the solidifying point of the fat is reached. If the sample is margarine, the fat can be collected into a relatively hard lump; but if it is butter, the mass is soft and creamy in consistence, or the fat is more or less distributed throughout the milk.

Margarine may be suspected if on burning a small portion of the substance on a clean platinum spatula, the peculiar odour of burnt tallow is given off, after extinguishing the flame.

When butter is heated in a platinum dish over a gas-burner it foams considerably, and may run over the dish; but there is less noisy spluttering than is commonly the case with margarine, and there is less foaming with the latter.

An alcoholic solution of sodic hydrate warmed with butter and then emptied on to cold water gives a distinct odour of pineapple; not so with margarine.

Admixture with *small* quantities of certain fats is practically unrecognizable by any of the tests already given.

Annatto, and more rarely turmeric, saffron, marigold, carrotin, and certain coal-tar colours, are used as colouring agents; their presence may be detected by shaking up about 5 grammes of the melted and filtered butter in a tube, with 25 c.c. of a mixture of 15 parts of methylic alcohol and 2 of carbon bisulphide; the fat dissolves in the carbon bisulphide, and the alcohol, along with the colouring matter, floats above. To ascertain the nature of the agent employed, special tests must be applied,

as indicated in the chapter on Antiseptics and Colouring Agents in Food.

Boric acid is frequently found in butter and cream, for it enters into the composition of proprietary nostrums sold for preserving these articles. Salicylic acid and sodium benzoate and fluoride have also been employed as preservatives of certain French butters.

Water is sometimes worked into butter in excessive quantities for fraudulent purposes, but water much exceeding 16 per cent. reduces the keeping powers of the butter. Most margarines contain a relatively small amount of water. Common salt is added to improve the flavour, and also to preserve the butter by checking the decomposition of the casein; rarely does the percentage amount exceed that which will lend a palatable amount of saltness to the butter—*i.e.*, about 5 or 6 per cent.

Butter occasionally contains the *Bacillus tuberculosis* and other acid-fast organisms; and bacteria determine its flavour.

CHEESE.

Cheese consists of the original constituents (chiefly the casein and fat) of the milk (cows or goats) from which it is made; but, as ripening proceeds, the sugar becomes transformed (chiefly into lactic acid), and the decomposition is accompanied by a con-

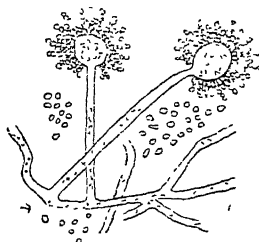



FIG. 38.—ASPERGILLUS GLAUCUS. (× ABOUT 200.)

siderable growth of bacteria, fungi, moulds, etc. There is no legal standard applying to cheese in this country.

There is comparatively little harmful *adulteration* practised in the manufacture of cheese. For the curd, which is separated from milk by rennet, there is no spurious and cheap substitute which can be made to yield the peculiar characters of pure cheese; but animal and vegetable fats are employed in the manu-

facture of "margarine cheese" and in the adulteration of the cheaper cheeses. The substance known as "filled cheese" is prepared from skimmed milk, lard, and other fats. Very little "margarine cheese" is sold in this country.

It has been shown that in some cases (especially of foreign cheeses) the surfaces have been brushed over with highly poisonous antiseptic solutions, such as arsenious acid and sulphate of copper, in order to preserve them; that colouring matters (lead chromate, etc.) have also been used to tint the rind; and that those small and delicate cheeses which are wrapped in thin lead

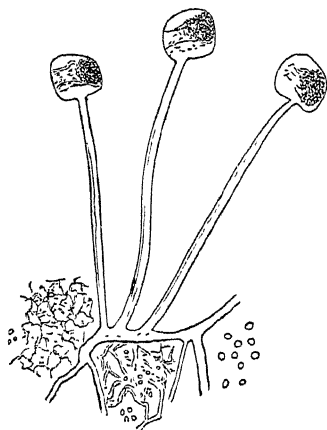



FIG. 39.—MUCOR MUCEDO. (× ABOUT 200.)

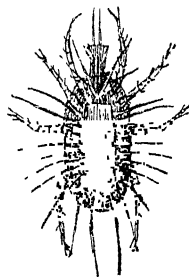



FIG. 40.—THE CHEESE MITE (ACARUS DOMESTICUS). (× ABOUT 40.)

papers may take up the metal. A careful examination, therefore, of the rind for metallic poisons may occasionally be desirable.

The proteid matter is ascertained by multiplying the nitrogen figure by 6·38.

The *fat* may be estimated by boiling a known weight of the dried cheese in a small flask with a quantity of strong hydrochloric acid. When it is all dissolved, the flask is cooled and the contents extracted with ether three times. The separated ether is now evaporated off from the flask to which it has been transferred, and the residual fat is dried and weighed. A deficiency of fat would be indicated, and suspicion warranted, if a cheese, other than Dutch cheese, contained less than 30 per cent. of fat.

The *purity* of the fat is ascertained by the Reichert-Wollny method of examination. The fat is easily extracted by boiling

some shredded cheese with strong hydrochloric acid in a flask, and then washing the fat in a separating-funnel with hot water.

In genuine cheese the Reichert-Wollny figure of the fat exceeds 18, whereas "margarine cheese" gives a figure which is generally below 6.

The action of ferments, etc., on the fat of cheese usually reduces the Reichert-Wollny figure; the "riper" the cheese, the lower this figure.

Cheese is peculiarly liable—and especially the moister kinds—to *parasitic growths*. *Aspergillus glaucus* is a form of vegetable fungus which gives rise to the appearance popularly known as "blue mould," and sometimes also to "green mould"; under the microscope its appearance is that denoted in Fig. 38.

Sporendonema casei is a similar growth, furnishing the appearance known as "red mould." *Mucor mucedo* (Fig. 39) is another fungus which attacks cheese.

Acarus domesticus, the cheese mite, is a tiny animal parasite shown magnified in Fig. 40.

The cheese maggots are animal parasites of much larger growth; they are the larvæ of a fly known as *Piophila casei*, and are readily detected by either the naked eye or a small hand lens.

LARD.

Lard is the fat obtained from the interior of the abdomen of swine; it is considerably adulterated with the oils and fats that are employed as butter substitutes (cotton-seed oil, cocoanut oil, beef stearin), and with excess of water.

The fats may be examined as in butter.

Paraffin has been found as an adulterant of lard. It may be detected by adding to 3 c.c. of the melted fat 10 c.c. of a mixture of equal parts of absolute alcohol and chloroform, and heating in a water-bath until complete dissolution. On cooling the tube with water, the contents become cloudy if paraffin is present.

CHAPTER V

CORN—WHEAT-FLOUR

CORN.

THIS term includes the seeds of cereal plants in general. Certain abnormal conditions of the entire seed which are brought about by small **animal and vegetable parasites** claim consideration.

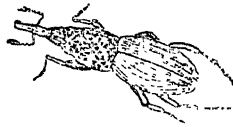



FIG. 41.—THE CORN WEEVIL (CALANDRA GRANARIA). (× ABOUT 40.)

Those seeds presenting minute round perforations, and consisting almost entirely of a shell, have generally been penetrated by a small insect, visible to the naked eye, termed *Calandra granaria* (*vide* Fig. 41), and popularly known as the "weevil."

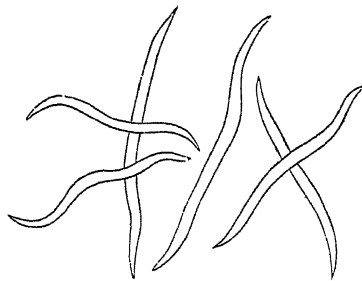



FIG. 42.—VIBRIONES TRITICI. (× ABOUT 40.)

These parasites may also be found in stored biscuits, to which articles species of Lepidoptera, notably the Ephestiæ, in different stages of development have sometimes proved very destructive.

Those which are small, black, or discoloured, and in which

the bulk of their substance is replaced by a fine cottony material, have been attacked by the "ear-cockle," or *Vibrio tritici*—a small worm-like parasite, pointed at either end as shown in Fig. 42. They are often to be seen in considerable numbers in damp wheat.

The *Acarus farinæ* is a small microscopic parasite which infests the grain, and closely resembles the *Acarus scabiei*. It especially

FIG. 43.—A WHEAT SPIKELET WITH EAR-COCKLE. (× 5.)

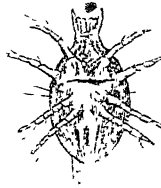



FIG. 44.—THE WHEAT MITE (ACARUS FARINÆ). (× 85.)

affects damp and inferior flour. Its characters are shown in Fig. 44. The eggs of the parasite are oval.

The various fungi which attack corn are the following:

1. *Claviceps purpurea* is a fungus chiefly affecting rye, and the mycelial growth which replaces the grain is known as *ergot*. In the spring of the year small hair-like growths with a knob at the

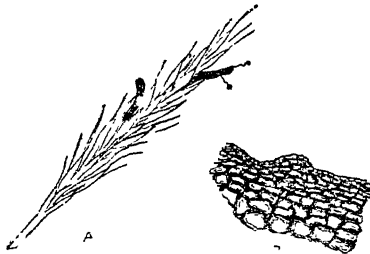



FIG. 45. (× ABOUT 250.)

A, Ear of rye with *Ergot*, the latter shown as germinating and producing *Claviceps purpurea* ; B, a section of ergot.

free end grow out from the mycelium; these are known as stromata, the microscopic appearance of which is shown in Figs. 45 and 46. Each stroma contains near its border a row of receptacles (ascocarps), containing onion-shaped bodies, known as asci. These asci ultimately get detached and rupture, liberating their contained eight filiform spores (ascospores), to

be borne by the wind to infect the ovary of the rye-flower. The fungus may cause a condition known as "ergotism" among many of those who habitually consume rye bread and biscuits. Those seeds which are not entirely replaced by the fungus are discoloured brown or purple, and the flour is also discoloured, and generally furnishes a peculiar sour odour. A microscopic

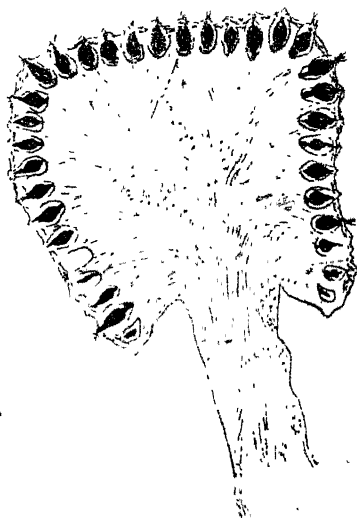



FIG. 46.—ERGOT.

Section of the end of a stroma, showing ascocarps and asci.

examination shows a very dense tissue formed by dark polygonal cells filled with oily constituents (Fig. 45), and to the naked eye the ovary is black externally and spongy internally.

Chemically, its presence in flour may be detected by the following methods:

(*a*) The flour is made into a paste with a weak solution of potassic hydrate, and then dilute nitric acid is added to slight excess. When the whole is subsequently neutralized by a little more of the potassic hydrate solution, a violet-red colour forms if ergot is present, and a violet colour is established when more of the alkaline solution is added.

(*b*) On the addition of potassic hydrate solution to the flour a distinct herring-like odour is appreciable—due to trimethylamine.

(*c*) The flour is made thoroughly moist with ether, a few drops of dilute sulphuric acid are added, and the whole is then well agitated; on the addition of a few drops of a saturated solution of sodium bicarbonate a violet colour appears (Hoffmann).

2. *Uredo segetum*, "smut," especially affects barley, rye, wheat, and oats. The fine dark dust, which sometimes gives the ear of wheat the appearance of having been placed up the chimney,

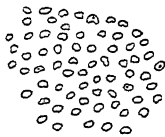



FIG. 47.—SMUT SPORES (UREDO SEGETUM). (× 200.)

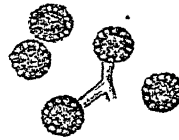



FIG. 48.—BUNT (UREDO FŒTIDA). (× 200.)

is inodorous, and has suggested the popular name "dust brand" to the condition. Bread made with flour thus affected is bluish. *Tilletia caries* (*Uredo fœtida*) and *Tilletia lævis* are of the same family (*Ustilagineæ*).

3. *Uredo fœtida* (*Tilletia caries*), "bunt," affects the interior of the grains of wheat, which it replaces by spores furnishing a

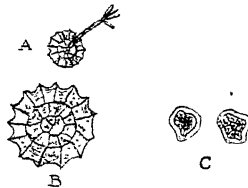



FIG. 49. (× 200.)

A, *Tilletia caries* (bunt), showing germination; B, bunt very highly magnified; C, *Tilletia lævis*.

fine dust, and hence the condition is sometimes called "pepper brand." The dust when rubbed between the fingers has a slippery and greasy feel, and gives off a peculiar fœtid odour. No ill effect has been ascribed to the consumption of flour affected with either *Uredo fœtida* or *Uredo segetum*. The microscopic appearance of "bunt" is shown in Figs. 48 and 49.

4. *Puccinia Graminis.*—The sporangia—as shown in Fig. 50—consist of dark, rounded masses, which show either a double linear contour or one presenting numerous small projections. The wheat-ear or barley and rye ears and stalk attacked by this

FIG. 50.—PUCCINIA GRAMINIS. (× ABOUT 200.)

fungus are more or less covered by a fine brownish deposit, which has been most aptly designated "rust."

5. *Mucor, aspergillus,* and *penicillium* may also be seen in decomposing corn.

WHEAT-FLOUR.

At the present day the miller endeavours to produce a flour which consists as nearly as possible solely of the contents of the

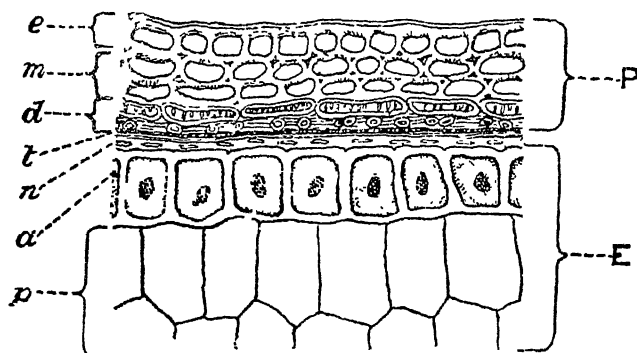



FIG. 51.—CROSS-SECTION THROUGH BRANNY ENVELOPE AND OUTER PORTION OF THE ENDOSPERM OF THE GRAIN. (× 250.)

P=pericarp, consisting of (*e*) epicarp, (*m*) mesocarp, and (*d*) endocarp. E=endosperm, consisting of (*a*) layer of aleurone cells (rich in protein, but free from starch), and (*p*) parenchymatous cells (packed with starch grains); (*t*) testa; (*n*) nucellus.

parenchymatous cells of the endosperm, and to this end the whole of the complicated machinery of modern milling is con-

trived. In practice, however, a perfect separation is never attained, and the flour always contains more or less of the other portions of the wheat grain, which are termed the "offal," consisting of the embryo or germ and the bran formed of the pericarp and the integuments and outermost layer of the seed. The inclusion of this offal raises the mineral, fat, and proteid content. But the oil in the germ is very prone to develop rancidity, and it is principally for this reason that it is rejected in modern milling. "Standard" flour is described as "80 per cent. of the wheat with all the germ and semolina," but this is unsatisfactory for more than one reason. In the first place, the term "semolina" does not connote any particular part of the grain; it is merely a trade name for the coarser fragments of endosperm produced in the break-roller system, and is therefore incapable of exact definition. In the second place, the requirement that the flour shall contain 80 per cent. of the wheat grain is by no means satisfactory as a "standard" of quality or composition. Wheats differ considerably from one another, and the skin or branny envelope bears a smaller ratio to the endosperm in the case of a large grain than of a small grain.

The composition of ordinary baker's flour and of "whole-wheat" flour varies with different samples, but the following results would fairly represent the average:

	Ordinary Baker's Flour.	"Whole-Wheat" Flour.
	(100 parts by weight).	
Protein (N=6·25)	12·3	13·8
Starch	71·2	68·1
Fat	1·3	1·9
Sugar	1·3	1·2
Fibre	0·4	1·7
Ash	0·7	1·4
(P_2O_5)	(0·21)	(0·67)
Moisture	12·8	11·9
	100·0	100·0

From the nutritive standpoint the increased protein of whole-wheat flour is discounted by the greater loss in the fæces, unless the wheat is very finely ground.

THE ANALYSIS.

Physical Characters of Flour.—The colour should be white, and the flour clean; a yellow hue denotes age or fermentation, and fermenting flour disarranges the digestive system, producing

flatulence, dyspepsia, diarrhœa, etc. There should be no acid or mouldy odour, and no taste of acidity or mustiness. Taken up in the fingers, the flour should be smooth and soft, with no lumpy or gritty feel; it should knit or bind together, and a little flecked on to the wall should mostly adhere; on mixing with a little water, the dough should draw out into stringy masses.

There must be complete freedom from fungi and other parasitic growths. If flour is stored in a damp place, the number of microbes present increases rapidly, and poisonous alkaloidal products may result from prolonged storage under such conditions.

As compared with other flours, wheat-flour is characterized by the large amount of **crude gluten** it contains; and it is to this substance—or rather to one of its constituents termed "gliadin"—that the peculiar adhesiveness of the flour, which makes it so peculiarly adapted for bread-making, is due. If flour is made into a dough with water, and then the dough is thoroughly washed, it is this crude gluten which remains behind as a sticky mass, the starch and soluble substances (*i.e.*, sugar, soluble albumin, and salts) being washed away. It is of great value, therefore, both as a test of the purity and also of the quality of the flour, to estimate the amount of this substance. One means of effecting this is the following:

Weigh out a quantity of flour—say 50 grammes—place it in a small basin, and carefully mix it with lukewarm water (about 16° C.) into the condition of stiff dough; then slowly and thoroughly work up the dough with the fingers, either under water, or while allowing a gentle stream of the warm water to fall upon it. As the dough becomes more and more washed, the water which is being constantly emptied away and renewed gets clearer and clearer, and the dough more stringy and sticky. Ultimately, the starch and all the soluble materials in the original flour are carried away, and the water escapes in a perfectly clear condition. Nothing then but crude gluten, containing generally a fraction over 1 per cent. of fats and salts, remains; and the entire absence of starch can be proved by treating with a little iodine. The gluten should then be spread out in a tared (weighed) flat-bottom dish, dried at 105° C., and finally weighed.

A more exact method of estimating the protein material is as follows:

THE ESTIMATION OF NITROGENOUS ORGANIC MATTER IN FLOUR (KJELDAHL'S PROCESS).

Special Reagents required.—(1) Decinormal sulphuric acid; (2) decinormal soda solution; (3) methyl-orange indicator; (4) strong sodium hydrate solution (500 grammes added to 500 c.c. distilled water, and well boiled to free from ammonia); (5) strong sulphuric acid, free from nitrates and ammonium; (6) red oxide of mercury; (7) potassium sulphate.

The Process.

1. Weigh from 0·5 to 3 grammes of the material (according to its richness in nitrogen), and transfer to a strong hard-glass boiling-flask.

2. Add 25 c.c. of strong sulphuric acid and 0·75 gramme of red mercuric oxide. Support the flask in a slanting position on a tripod, and by means of a Bunsen burner keep the acid just below its boiling-point for half an hour. As fumes of sulphuric acid will escape from the mouth of the flask, the heating must be done in a fume-cupboard.

3. If at the end of half an hour the mixture is still black, 12 grammes of potassium sulphate (free from nitrates) are added. This raises the boiling-point, and the heating is continued for a few minutes after the liquid is clear and has no more than a faint yellow tint.

4. Let cool; add about 500 c.c. of ammonia-free water, and be careful to wash thoroughly the mouth and neck of the bottle with this water. Then more than neutralize the acid by means of the strong soda solution. Also add about 20 c.c. of a 4 per cent. solution potassium sulphide, in order to precipitate all the mercury as sulphide, and thus prevent the formation of mercurammonium compounds.

5. Distil over the ammonia into 50 c.c. of decinormal sulphuric acid, using a condenser with a bulb ("anti-splasher") in the condensing-tube a little above the boiling-flask, in order to guard against the liquid spurting over.

6. When about 250 c.c. of distillate have been collected, the acidity is titrated with the decinormal soda solution, using methyl-orange as indicator. The difference between the amount of soda solution required to neutralize the 50 c.c. of decinormal acid, before and after the addition of the distillate, represents the ammonia which has come over. Each c.c. of the acid

neutralized by the ammonia=0·0014 gramme of nitrogen, and the nitrogen multiplied by the factor 5·68 (as the protein of wheat contains an average of 17·6 per cent. of nitrogen) represents the amount of albuminoid or protein material in the amount of the flour examined.

Notes on the Process.—The organic matter is burnt up in this process by moist combustion, and the resulting ammonia combines with the sulphuric acid to form ammonium sulphate. The addition of excess of soda liberates the ammonia from the acid and enables it to be distilled over. It is necessary to perform a blank experiment occasionally in order to test the reagents. The figure of the blank experiment (commonly only about 0·2 c.c. of decinormal alkali) should, of course, be deducted in arriving at the total nitrogen obtained from the material.

Example.—Two grammes of flour furnished ammonia which neutralized 19·5 c.c. of the decinormal acid.

∴ there are 19·5×0·0014=0·0273 gramme of nitrogen in 2 grammes of flour=1·365 grammes of nitrogen in 100 grammes of flour.

∴ 1·365×5·68=7·75 per cent. of protein material, or approximately 7·7 per cent. of gluten.

The gluten varies from 8 to 12 per cent.; if the gluten is less than 8 per cent. the flour is not pure wheat-flour; and if it does not amount to 10 per cent. and cannot be drawn out into long fine threads without breaking, the flour is poor in quality. Rye yields a plastic gluten which cannot be separated by washing.

The **water** of flour should not exceed 15 per cent. by weight, since more than this, besides fraudulently throwing up the weight, impairs its keeping power by favouring the development of fungi and the acetic and lactic acid fermentations, which may sometimes produce gastro-intestinal disturbance. The amount of moisture is, of course, ascertained by drying a weighed quantity of flour over the water-bath (and subsequently in the hot-air oven), the loss in weight being due to moisture.

The **ash** of wheat consists chiefly of phosphates of potassium, magnesium and calcium, and small quantities only of salts of silica, sodium, iron, etc.; the amount should not reach 1 per cent., and as much as 2 per cent. would imply that mineral adulterants have been added. In making the estimation, cautiously incinerate a weighed quantity of dried flour in a platinum dish, until a clean white ash remains. During ignition

a hard mass of carbon forms, and it is a good plan to moisten this with a strong solution of nitrate of ammonia, then dry and continue the ignition. It may be necessary to repeat this treatment before a clean ash is obtained.

Adulteration.—*Foreign* mineral matter, which is seldom now added, may be roughly estimated by shaking up with chloroform, when the flour floats and most of the added mineral matter settles at the bottom of the vessel. The treatment is repeated, in order that it shall be as inclusive as possible, and the sediment collected, dried, and weighed; it can also be examined as to its nature.

The presence of any added mineral matter (calcium phosphate, sulphate, and carbonate, etc.) is also readily detected in the ash, as this is found to be exceptionally high.

Copper sulphate, probably employed to prevent or destroy fungoid growth in the corn, has been detected in flour and bread. Soakage in water to which a little potassium ferrocyanide solution and a drop or two of acetic acid has been added will detect this by the appearance of a coppery tint.

Phosphates and other "improvers" are employed to improve the baking quality of certain flours by improving the nature of the gluten, and thus increase the strength and water-absorbing capacity of the flour.

A mixture in about equal proportions of acid potassium and magnesium phosphates and flour is said to be an effective improver.

The use of calcium acid phosphate has greatly extended in recent years, and as this substance may contain a large proportion of calcium sulphate, which is valueless, Dr. Hamill, in a Report to the Local Government Board (1911), recommends that a maximum limit of 10 per cent. of calcium sulphate in calcium acid phosphate should be fixed.

There is no evidence that these phosphatic improvers increase the organic phosphorus compounds present; they are added with the object of increasing the amount of bread which can be obtained per sack of flour.

Rarely it is by the addition of other flours and meals that sophistication is practised, when, of course, the cheaper varieties are selected, such as maize. To detect rice-starch in wheat 33·33 grammes of flour are made into a ball with 17 grammes of water, and worked between the fingers in a fine stream of water

over a fine-meshed sieve. The starch and waste water (thus separated) are well shaken, and set aside for twelve hours in a large conical flask, when the starch separates in three well-marked layers, which can be separated by decantation. The top layer contains most of the small starch granules, and the bottom layer the largest grains, whereas the middle layer is mainly composed of the cellulose and proteid element of the flour. When rice-starch is present, it is almost entirely deposited in this layer, and its presence can be detected in so small a proportion as 1 per cent. (E. Collin). Maize is difficult to detect; but if the flour is mixed with clove oil the hilum of maize appears under the microscope as a black star or spot. This is not the case with the other starches most commonly used to adulterate flour.

Old flour is occasionally passed through the mill with fresh flour; in these cases there is marked acidity, a reduction in the fat and in the quality of the gluten.

The degree of whiteness of flour depends upon the fineness of the grade, and to some extent upon the variety of the wheat; and the artificial means of producing a white flour is by means of bleaching with nitrogen peroxide gas (NO_2). The bleaching effect appears to be due to the destruction of a yellow colouring matter dissolved in a thin layer of oil which surrounds each granule of starch. Dr. Hamill, reporting upon this matter to the Local Government Board, states that the practice cannot be regarded as free from risk to the consumer, especially when regard is had to the inhibitory effect of the bleaching agent on digestive processes. This bleaching of flour is prohibited in Switzerland, United States, and certain of the Australian States. The amount of nitrite left in the flour is extremely minute, and in the bread made from the flour it is still further reduced; and for practical purposes it may be presumed that when over 1·5 parts of nitrites per million are present, the flour has been bleached. Small amounts of nitrate are also formed as a result of bleaching. Extremely small quantities of nitrites can be tested by the Ilosvay method, by which the colour produced with sulphanilic and *a*-naphthylamine hydrochloride in acetic acid solution may be compared with standards containing known amounts of sodium nitrite.

The bleaching of flour by chemical oxidizing agents has been introduced in response to the public demand for a white loaf.

A simple test for bleached flour is to shake up about ½ ounce of the flour with 2 fluid ounces of petrol. If unbleached, the spirit takes up a yellow colour, but not so if the flour has been bleached. The bleaching of flour by means of nitrogen peroxide renders the gluten indigestible (Halliburton).

Dr. MacFadden, in a Report to the Local Government Board, draws attention to the fact that the relation which may exist between apparently very minute alterations in the nature of staple food materials and the production of great and far-reaching changes in nutrition has been strikingly demonstrated in recent investigations into certain obscure disorders of metabolism, of which the disease known as beri-beri may be taken as an example, and the time has arrived for taking a wider view than has hitherto been customary of the danger to health which may arise from the sophistication of foodstuffs.

The seeds of the darnel grass, or *Lolium temulentum*, may gain access to wheat or oat flour; they are said to possess narcotic poisoning properties. Neither the starch grains nor the testa are characteristic under the microscope, since both resemble oats very closely; but the addition of alcohol causes a greenish colour to appear, together with a peculiar repulsive taste, if flour contains these seeds.

The corn-cockle (*Agrostemma githago*) consists of large, dull black seeds, showing small protuberances. They are markedly poisonous.

CHAPTER VI

BREAD

It is only with wheaten bread that the following chapter deals.

Among the means for obtaining the porosity of bread is included the use of **baking-powders.** These consist most generally of a mixture of sodium bicarbonate, tartaric acid, and rice-flour. The rice-flour is used to keep the powder dry, and to prevent chemical action setting up until it is moistened.

The tartaric acid baking-powders are by far the most common, but powders are also sold in which the acid constituent is furnished by acid phosphate, and in other cases by the sulphuric acid contained in some form of alum salt. It has been argued that the employment of acid phosphates is of value as replacing the phosphates lost to the bread by the removal of the bran.

Certainly the use of baking-powders containing alum should be condemned. The reaction between potassium-alum and sodium bicarbonate has been shown to result in the production of aluminium hydrate, and the hydrate of alumina is known to be dissolved (with difficulty) by the gastro-intestinal juices. Such a baking-powder, analyzed by the writer, gave 23 per cent. sodium bicarbonate, 33 per cent. alum, and 44 per cent. ground rice, etc.

Self-raising flour is flour containing the essential elements of baking-powder.

In the process of cooking, some of the starch of the wheat-flour is converted into maltose.

The composition of good bread (freed from moisture) is approximately as follows:

Starch, dextrin, etc.	82·6
Nitrogenous matter	11·4
Maltose	4·0
Fat..	0·6
Salts	1·4
	100·0

Physical Characters.—The bread should be fairly dry, light, and spongy; and not sodden, acid, or musty. It should be clean and of a good colour—nearly white, that is to say; for a yellow or dirty colour betrays age and poorness in quality. A peculiar violet tint is given to wheat containing *melampyrum* and other species of Scrophulariaceæ and *trefolium* (trefoil). Other growths sometimes give the bread a dirty blue appearance (*rhinanthus*, etc.); *agrostemma* (corn-cockle) furnishes a greenish tint. *Oidium aurantiacum* has caused poisoning in France. It is a reddish-yellow mould, giving a bitter taste and offensive odour to the bread.

Viscous fermentation, more frequently to be observed in milk, beer, and wine, occasionally produces "ropy bread." The causal organism is most probably a variety of *B. mesentericus*. In the centre of the loaf a brown glutinous mass appears, with a nauseous odour. The organism, which is in the flour or gains access during the mixing of the dough, has been called (Vogel) *B. viscosus panis*; and if a "ropy" loaf be broken, small glistening colonies may be observed with the naked eye. Such bread is unfit for human consumption, although no harmful effects have been recorded.

The estimation of **water and mineral matter** is performed as in flour. Fifty grammes of crumbs is a convenient amount to work with.

The moisture in bread should not much exceed 40 per cent. in the crumb part and 25 per cent. in the crust.

The Ash.—An increase in weight of the ash of bread over that of the original flour is due to the common salt and the baking-powder which are added in the process of baking. But any excess of ash above 3 per cent. would be due to added minerals (such as gypsum and chalk), which have been rarely added with the object of improving the colour.

As chlorides and other salts may be volatilized by the prolonged ignition necessary to furnish a white ash, the ash must be procured at as low a temperature as possible.

To estimate the silica, treat the ash first with strong hydrochloric acid, then with a little distilled water, and boil; next filter through a Swedish filter-paper, wash the platinum dish by boiling more distilled water in it, and filter these washings also through the same paper. When the platinum dish is perfectly clean, well wash the material upon the filter-paper with small

quantities of hot distilled water; dry in the water-oven, and then ignite in a porcelain crucible with lid; finally weigh the ash; deduct the weight of the filter-paper ash, and the difference is silica. It should not exceed 0·2 per cent.

To estimate **acidity,** soak 10 grammes of bread in about 50 c.c. of water for one hour, filter, and titrate the filtrate with a decinormal alkaline solution, using phenolphthalein as indicator. The number of c.c. of decinormal soda used to neutralize the acidity × 6=milligrammes of glacial acetic acid in 10 grammes of bread. Express results in terms of glacial acetic acid per cent.

Anything over 0·12 per cent. is rather acid, and this figure should, therefore, not be exceeded.

Adulteration.—Mashed potatoes are looked upon as a legitimate addition in slight amount where sponginess is dependent upon fermentation, since they favour this action. It has been said that they are added to increase the weight and whiten the loaf, and since they contain between 70 and 80 per cent. of moisture, they help to keep the bread moist; but generally only the strained liquor in which the potatoes have been cooked and mashed is employed, in order to obtain a sweeter loaf.

Potato starch takes up certain coal-tar colours much more rapidly than starches derived from wheat and rye, and its presence may therefore be readily detected microscopically in a suitably stained preparation. The procedure is as follows: A few breadcrumbs are disintegrated by moistening with water and gently pressing with a cover-slip. The preparation is then dried by rapidly passing the slide through the flame, and a drop of an aqueous solution of neutral-red or methylene-blue is applied for a definite time, varying from one and a half to three minutes, according to the dye. Excess is then washed away, and the stained specimen examined under a low power. With neutral-red, wheat and rye starch grains appear colourless, and potato starch rose-red, and a similar differentiation exists with methylene-blue.

Rice, when added, also serves the purpose of giving a good white colour to the loaf.

Dr. Alford has recorded an outbreak of lead-poisoning, affecting from fifteen to twenty persons, arising from the consumption of flour which had been ground by an old millstone in which large spaces had been filled in with lead.

The starch grains of the flour used in the manufacture of

bread become so altered by the process of cooking (on account of the rupture of their envelopes) as to lose most, if not all, of their microscopic characteristics.

Fungi may be discovered, and notably the different forms of *penicillium* ("mildew"); these may create, if sufficiently numerous, patches of greenish, brownish, or reddish discoloration. *Oidium aurantiacum* furnishes an orange hue. These fungi should condemn the bread off-hand, for they may give rise to considerable gastro-intestinal disturbance.

Alumina exists normally in pure flour as the silicate of alumina; but it has been added, as **alum,** to inferior flour, so as to check the fermentative action, whereby a large amount of sugar (glucose) is formed, and a discoloured bread, unpleasant

FIG. 52.—PENICILLIUM GLAUCUM. (× ABOUT 200.)

to the palate, results. Alum thus improves the taste and colour of the bread, and also to some extent its porosity.

The whitening of flour has also been obtained by bleaching methods, so that the colour is no certain indication of quality.

At the present day, largely owing to the adoption of other expedients, alum is very little employed, and it is rare that alumina is detected in amounts which denote an excess over that which may be *normally* present. Large quantities of the salt were formerly added to flour, and the great decline in its use commenced with the passing of the Sale of Food and Drugs Act, 1875. It was generally employed in quantities of about 15 to 35 grains to a 4-pound loaf, but more than 100 grains have been separated.

It is generally held in this country that *no* addition of alum should be countenanced, and that very small amounts in an article such as bread, of which large quantities are consumed, may prove deleterious to health, by inducing dyspepsia, constipation, etc. There is, however, some conflict of opinion as to

whether small quantities of alum are injurious to health; but there can be no doubt that it is an adulteration under the Bread Acts of 1822 and 1836.

The best **test for the presence of alum,** and one which will detect as little as 1 grain per pound in bread which has not undergone acid fermentation, is as follows:

Reagents required.—1. A strong freshly-made tincture of logwood, prepared by digesting 5 grammes of freshly-cut logwood chips in 100 c.c. of strong alcohol.

2. A solution of ammonium carbonate (15 grammes of ammonium carbonate in 100 c.c. of distilled water).

About 5 c.c. of each of these reagents are added to about 30 c.c. of water, and pieces of the crumb of the bread are cut from the loaf, moistened with a little water, and left to soak in this mixture for a few minutes; the fluid is then drained off and the bread gently dried over the water-bath. The presence of alum is denoted by the appearance of a permanent lavender or violet colour, according to the amount present; while the parts of the bread which contain *no* alum are first stained the bright colour of the logwood solution, and afterwards change to a dirty brown tint. Wynter Blyth soaks the bread paste in gelatine, and then tests this with logwood and ammonium carbonate; a neater reaction is thereby obtained.

The operator must be careful that he is not led astray by magnesium salts, which are capable of creating a lavender tinge almost identical with that of alum; but the colour created by these salts is certainly not so permanent upon drying as that furnished by alum.

In order to avoid the effect of acidity of old meal or of sour bread on the logwood test, the following method is recommended: From 10 to 20 grammes of the bread are triturated into a paste with water, some sodium chloride (free from alkali) added, and then, after the addition of 10 drops of freshly prepared logwood tincture, 5 grammes of pure potassium carbonate are gradually mixed in. After being well mixed, the whole is washed with 100 c.c. of water in a beaker and allowed to settle. In a few minutes the supernatant liquid becomes a greyish to a deep blue when alum is present, and a reddish-violet tint when it is absent.

As a confirmatory test (Herz) for alum in flour, 10 grammes of the flour are mixed with water and allowed to stand for

ten minutes; filter, concentrate, and precipitate the protein with tannic acid solution. Filter and add 2 drops of tincture of cochineal. In the presence of alum a carmine red colour is obtained.

Conclusions to be Drawn from the Amount Estimated.—If the amount of alumina represents more than from 6 to 10 grains of alum per 4-pound loaf, in the vast majority of cases the latter has been fraudulently added; some pure flours, however, may undoubtedly contain a greater quantity than this, and hence it is difficult to lay down any definite quantity as a standard beyond which the proof of fraudulent addition may be certainly established. The alumina which is taken up from the soil is in the form of silicate, and if the amount of alumina considerably predominates over that of the silica, that circumstance would denote the presence of "added alum."

CHAPTER VII

THE AVERAGE COMPOSITION OF OTHER FLOURS AND MEALS* —THE MICROSCOPIC CHARACTERS OF THE DIFFERENT STARCH GRANULES

OATS.

Starch, dextrin, and cellulose	64·5
Nitrogenous matter	12·0
Fat	6·0
Mineral ash	3·0
Sugar	2·0
Water	12·5
	100·0

SAGO.

Starch, dextrin, and cellulose	86·0
Nitrogenous matter	0·8
Mineral ash	0·1
Water	13·1
	100·0

CORNFLOUR† (MAIZE).

Starch, dextrin, and cellulose	68·5
Nitrogenous matter	13·0
Fat	3·5
Mineral ash	1·5
Water	13·5
	100·0

PEA.

Starch, dextrin, and cellulose	58·5
Nitrogenous matter	23·0
Fat	2·0
Mineral ash	2·5
Water	14·0
	100·0

ARROWROOT.

Starch, dextrin, and cellulose	83·0
Nitrogenous matter	0·8
Mineral ash	0·2
Water	16·0
	100·0

TAPIOCA.

Starch, dextrin, and cellulose	87·3
Nitrogenous matter	0·6
Mineral ash	0·1
Water	12·0
	100·0

LENTILS.

Starch, dextrin, and cellulose	58·5
Nitrogenous matter	25·0
Fat	2·0
Mineral ash	2·5
Water	12·0
	100·0

BEAN (HARICOT).

Starch, dextrin, and cellulose	57·5
Nitrogenous matter	23·5
Fat	2·0
Mineral ash	3·0
Water	14·0
	100·0

* Chiefly compiled from the results of analyses made by the writer.
† Cornflour consists of the nearly pure starch of maize or rice.

RYE.

Starch, dextrin, and cellulose	68·0
Nitrogenous matter	11·0
Fat	2·0
Mineral ash	1·5
Sugar	3·5
Water	14·0
	100·0

POTATO.

Starch, dextrin, and cellulose	22·0
Nitrogenous matter	2·0
Fat	0·1
Mineral ash	1·0
Water	74·9
	100·0

BARLEY.

Starch, dextrin, and cellulose	71·0
Nitrogenous matter	11·5
Fat	1·5
Mineral ash	1·0
Water	15·0
	100·0

RICE.

Starch, dextrin, and cellulose	78·5
Nitrogenous matter	6·5
Fat	0·5
Mineral ash	0·5
Water	14·0
	100·0

A silicate of magnesia (talc) is sometimes employed to polish or "face" rice, in order to improve its appearance; oil may be employed to increase translucency; and blue pigments (ultramarine) to improve the white colour.

The more expensive of these flours, or meals, are liable to be adulterated with the cheaper kinds, such as rice, tapioca, potato, and maize.

It will be seen that, in comparison with wheat, barley is poor in nitrogenous matter and sugar, but rich in cellulose and mineral matter; that oats are exceptionally rich in cellulose and fat, possess a high amount of mineral matter, but are relatively poor in starch; that maize possesses a high amount of fat, but the cellulose is low; that rye is exceptionally rich in sugar, and in other respects closely approximates to wheat; and that rice is rich in starch, but poor in everything else.

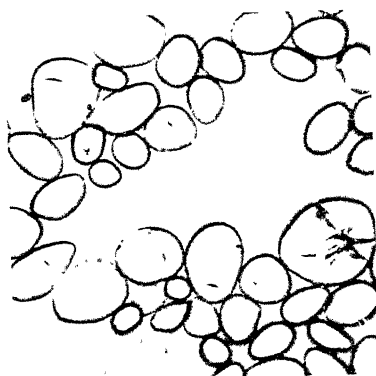
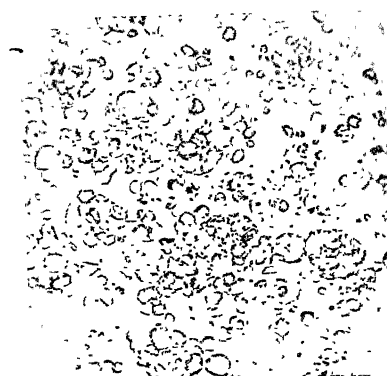
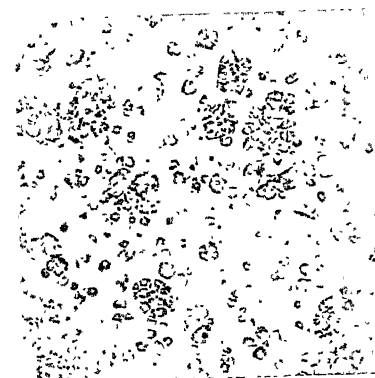
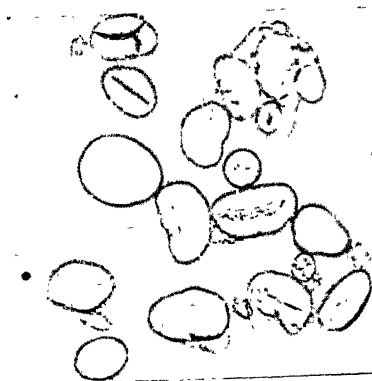
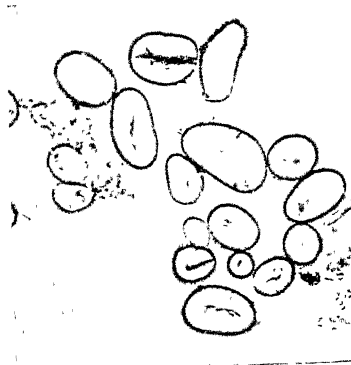
The amount of *starch* in any substance is estimated as follows: Five grammes of the dried and powdered material are mixed with 200 c.c. of 4 per cent. HCl, a reflux condenser is attached to the flask, and the liquid is boiled for five hours. The contents are then cooled, made slightly alkaline with sodic hydrate, and the dextrose estimated by Fehling's method. The dextrose $\times 0·9 =$ starch. If cellulose is present, a little of this would also be converted into sugar by boiling with the acid, but this small quantity is often ignored.

The Microscopic Characters of the Different Starch Granules.

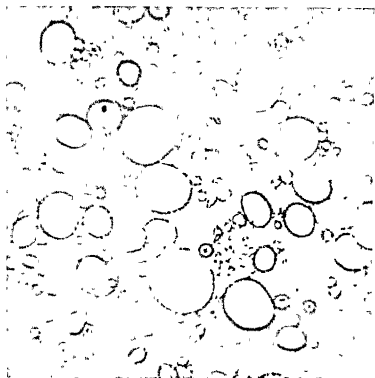
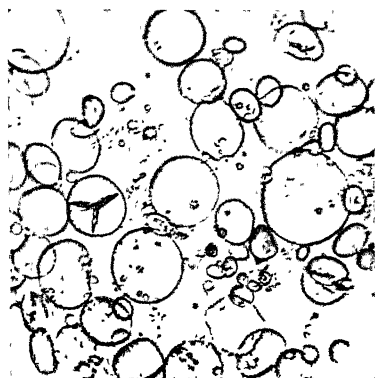
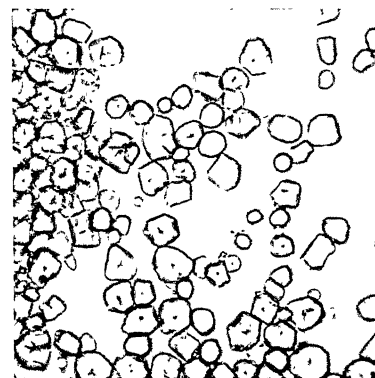
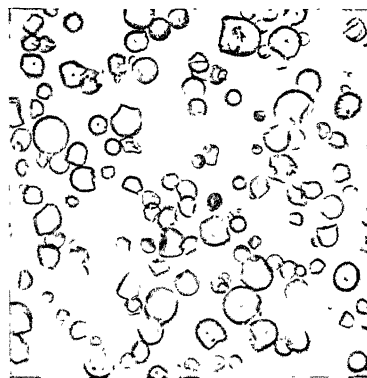
The starch of which the foregoing foodstuffs are mainly composed exists in the form of microscopic granules, which are more or less characteristic of the particular plant from which they are derived, on account of their difference in size, shape, and markings. These microscopic granules consist of an extremely thin envelope of cellulose enclosing the starch (granulose), and the latter appears to be generally arranged in fine superimposed strata—which accounts for the "striæ," or concentric lines, commonly discernible upon the external surface of the granule.

When a sample of any flour or meal is to be examined under the microscope, very small amounts are placed upon several clean glass slides, a drop of water is applied to each slide, and a clean cover-glass is pressed firmly down over the powder and water to evenly distribute the powder. It is impossible to get *too thin* a layer of the substance in order that a satisfactory examination may be made, as otherwise granules get superimposed and conglomerated, and their contours and markings cannot be defined. It is a good plan, therefore, to drop a small amount of the powder upon the slide, and then to gently blow almost all away again, before applying the water and cover-glass.

It is important that the reader should recognize that in the description which follows the most characteristic features are described. It must not be thought that in a sample of arrowroot, for instance, each granule will possess the characters described under that head. Such is by no means the case, for some may have the hilum in the centre, or even at the small extremity of the granule (as in potato), and yet the sample may be pure; but many of the granules will possess, in a more or less marked degree, the characters described. Where, therefore, the starch grains of different food-plants somewhat closely resemble each other it is difficult to decide as to whether there may be some slight admixture, although considerable adulteration admits of no questioning; but when these grains are dissimilar in appearance, the faintest possible amount of admixture is readily detected. When it is required to estimate the *amount* of adulteration, a rough percentage of the foreign starch grains present may be made by counting them upon the microscopic

PLATE V.

STARCH GRANULES. (× 250)

E C Bousfield, pho

PLATE VI.

STARCH GRANULES (× 250)

E. C. Bousfield, photo.

"field" of several mounted specimens. When the percentage amount of foreign starch has been estimated, a careful and thorough mixture is made up containing the supposed amounts of the ingredients in the composition under examination; this is then examined under the microscope, and the counts compared with those of the original powder, in order to see if the estimation which has been made is broadly correct. If not, known quantities of the pure substance are mixed with fresh quantities of the adulterants found until a microscopic examination shows that the approximately true percentages have been arrived at. In cases where the foreign starch granules are very distinctive, the number in the specimen may be counted upon a plan similar to that adopted in the case of counts of blood-corpuscles.

It will be seen that, in many cases, the differences between the starch granules are very slight, and therefore some skill is requisite in detecting them; such skill is only acquired from practice, and the student is recommended to fit up a small case containing samples of all the more common starches and to practise assiduously with these. Specimens mounted in glycerine are well preserved for a short time. A ¼-inch power should be employed, and this suffices for all practical purposes.

Mention may be made of the useful adjunct which the polariscope may furnish to such investigations. For polariscopic examination glycerine or oil should be used instead of water. Starches, such as potato, arrowroot, bean, and maize, polarize well; while wheat, rice, and oatmeal polarize feebly.

1. *Large round or oval granules, more or less flattened, and showing no marked concentric "striæ" (or at most only a few at the margins), together with other granules extremely small and ill-defined.*

May be wheat, barley, or rye.

Wheat.—Relatively few "intermediary"* sizes, although the larger granules themselves vary somewhat in size. (A linear hilum and striæ are visible under a very high power, and the small granules are seen to be angular.)

Barley.—Similar; but the large granules are rather more irregular in shape and somewhat smaller, and "intermediary" sizes are more commonly present; lumpy forms rather more common.

* A term used, in this connection, to denote a size about *midway* between that of the large and small granules.

Rye.—Similar; but many show a rayed hilum, and present cracked edges; the granules are somewhat larger, and more generally circular and flattened than those of wheat or barley. Striations often distinct. Rye-flour is darker and less finely ground than wheat-flour.

2. *Large pyriform or oval granules, with well-marked concentric striæ and a circular or short linear hilum.*

May be potato or arrowroot.

Potato.—Typically, a well-marked circular or stellate hilum is at the smaller extremity, and the striæ are well marked. The granules vary considerably in size.

Arrowroot.—Similar; but the hilum is generally at the larger extremity, and the granules average a trifle smaller (with the exception of the arrowroot named "tous-les-mois," in which commonly the granules are even larger than those of potato, though they vary considerably in size). The granules do not swell with potassic hydrate solution, as do those of potato, and the

FIG. 53.—BRUCHUS PISI (OF THE PEA, BEAN, ETC.). (× ABOUT 40.)

concentric rings are, generally speaking, less visible. There are many varieties of arrowroot, all of which present similar general characteristics as to their starch granules; the common variety is derived from *Maranta arundinacea.*

3. *Oval or reniform granules, with faint concentric striæ, a central linear hilum, and very uniform in size.*

May be pea or bean.

Pea.—Most have a central longitudinal hilum, which presents a puckered appearance. The granules are large.

Bean.—Similar; but somewhat larger and more flattened (*i.e.*, broader), and slightly more uniform in size. The hilum is much more commonly crossed by transverse lines ("puckered").

4. *Very small angular and faceted* granules, without concentric striæ.*

May be rice, oatmeal, or maize.

Rice.—The minute granules tend to collect into angular masses.

* These facets are due to the close juxtaposition of the granules.

Oatmeal.—The granules tend to collect into rounded masses, and are slightly larger than in rice, but still very minute.

Maizê.—The granules are much larger and are more irregular in shape, which tends towards the circular; they possess a visible hilum which is generally stellate.

5. *Irregular in size, rounded, or partly angular with rounded edges, possessing (generally) a central hilum, and occasionally showing ill-defined concentric striæ.*

May be sago or tapioca.

Sago.—Mostly large and irregular in shape; many elongated, with one larger end rounded and the other truncated. Hilum stellate or linear.

Tapioca.—Similar; but much smaller, and many granules have a tendency to be truncated by one facet. Hilum generally more towards the rounded extremity.

FIG. 54.—SECTION OF WHEAT GRAIN (OUTER COAT). (× 50.)
a, Girdle cells; *b*, cerealin cells.

In order to get rid of starch, oleo-resin, etc., and thus bring together within a small compass much of the vessels, fibres, and parenchyma, the following steps are serviceable:

Five grammes of powdered material are mixed with 50 c.c. of water to which 2 c.c. of HCl (S.G. 1·16) are added. The mixture is boiled for ten minutes, and then centrifugalized; the solid matter is washed, partially dried, stirred with a few c.c.'s of chloral hydrate solution; and the mixture is again centrifugalized and the deposit examined under the microscope.

The flours and meals from cereals also give evidence under the microscope of the thin envelope of tho grain, called the skin or testa; and this is the case with even the finest ground and purest flours.

In *wheat* the envelope is composed of three* fine membranes, the external and the middle both consisting of flattened cells, which are more or less dovetailed into each other.

The long axes of the cells in the middle coat are disposed at right angles to those in the external, the latter being arranged with their long axes corresponding with that of the grain.

Unicellular cells with pointed apices ("hairs") come off in tufts from the external coat at one extremity of the grain; these "hairs" are simply prolongations of the cells.

The internal coat is made up of irregularly rounded, opaque-looking cells, which frequently contain one or more oil globules. The starch granules, comprising almost the whole of the interior of the grain, are included within a thick-walled cellular network.

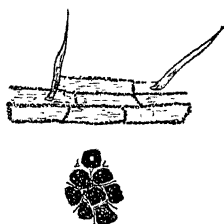



FIG. 55.—WHEAT. TISSUE FROM THE "TESTA" OF THE GRAIN, SHOWING THE APPEARANCE OF THE CELLS FORMING ITS OUTER AND INNER MEMBRANES. (× 100.)

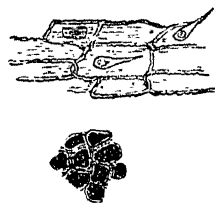



FIG. 56.—BARLEY. TISSUE FROM THE "TESTA" OF THE GRAIN, SHOWING THE APPEARANCE OF THE CELLS FORMING ITS OUTER AND INNER MEMBRANES. (× 100.)

In *barley* the envelopes are the same as those in wheat, except in the following respects:

The cells forming the external coat are shorter and more uniform in size than in wheat, and their outline is serrated instead of beaded; they carry, moreover, *short thick* hairs. The cells of the middle coat are more elongated, and those of the inner coat are somewhat smaller.

Slight as these differences are, it is to the envelopes rather than to the starch granules that one must turn in order to discriminate between wheat and barley.

In *rye* the testa so closely resembles that of wheat that it is difficult to hit upon a point in which they differ, and it is fortunate that the starch grains afford a ready means of distinguishing between the two.

* There are probably six in all under very high powers.

It may be pointed out that the unicellular hairs are somewhat shorter than in wheat.

In *maize* (Indian corn) the envelopes are two in number; the external consists of several superimposed layers of flattened, elongated cells, and the internal of a layer of cells of irregular size and shape, but otherwise resembling the internal layer of wheat. A very characteristic circumstance about maize is that the cellular network which holds the starch granules in this plant forms an irregular mosaic, most often pentagonal, but occasionally polygonal in design.

Spoilt maize taken as food may be responsible for pellagra, which is probably a food intoxication induced by some toxicogenic saprophyte. Many varieties of parasite are found on maize, including certain moulds, the spores of which are not destroyed by cooking.

FIG. 57.—RYE. TISSUE FROM THE "TESTA" OF THE GRAIN, SHOWING THE APPEARANCE OF THE CELLS WHICH FORM ITS OUTER AND INNER MEMBRANES. (× 100)

FIG 58.—OATS. TISSUE FROM THE "TESTA" OF THE GRAIN, SHOWING THE APPEARANCE OF THE CELLS WHICH FORM ITS OUTER AND INNER MEMBRANES. (× 100.)

In *oats* the envelopes consist of an external one of long narrow cells with evenly serrated contours (not wavy or beaded), and carrying sharp spinous "hairs"; a middle, somewhat similar coat, but indistinct and poorly seen; and an inner layer of cells resembling the internal one of wheat, but larger.

In *rice* the external coat of the husk, consisting of long narrow cells, is characterized by the number of fine silicious particles it contains, which are collected together into ridges, crossing each other at right angles. The spinous hairs are long and numerous, and the other coats, of which there are several, consist also of elongated, narrow, flattened cells, variously arranged.

The "polishings" from white rice appear to contain a sub-

stance the absence of which may lead to beri-beri when white rice is the staple food.

The polishing of rice results in the removal of the pericarp and of the whole of the greater part of the sub-pericarpal layers of the rice grain. C. Funk has pointed out that a satisfactory measure of the degree of polishing to which rice has been subjected is the estimation of its total phosphorus, and that a rice which yields less than 0·4 per cent. of P_2O_5 cannot safely be permitted to form the staple diet of man.

CHAPTER VIII

MEAT—PARASITES OF FLESH—POISONING BY FOOD—MEAT PREPARATIONS

IT is sometimes necessary to make a laboratory examination of meat, in order to decide whether it is fit for human consumption.

THE CHARACTERS OF GOOD MEAT.

It should have a marbled appearance, due to little streaks of fat between the muscular fasciculi; the whole surface should have a glossy appearance, and the colour should be of a bright florid hue and not too dark, or the meat is that of an old or diseased animal. The colour of veal, mutton, and pork is always paler than that of beef, and this fact depends to some extent upon natural causes (the flesh of all young animals is naturally paler than that of older ones), but mostly upon the fact that calves, sheep, and pigs are bled more at the time of killing. In old animals the flesh is darker and tougher, and the fat more yellow and soft.

The connective tissues should glisten when exposed, and the muscular fasciculi should not be too large and coarse.

To the touch the meat should be firm and slightly elastic, which implies that the meat is fresh and has set well (rigor mortis); it should, moreover, be so dry upon the surface that the finger is only slightly moistened by being passed over it; such moisture should be of a clear red colour and of an acid reaction. In taking the reaction the litmus-paper should first be dipped in water, as otherwise the serum glazes the paper and obscures the reaction. On cutting through the flesh, the whole thickness should present a uniform colour, or the interior must be but very slightly paler than the more external flesh.

The odour of meat is best obtained either by drenching it

(when finely minced) with very hot water, or by plunging a clean odourless knife or new wooden skewer deep down into its substance—preferably in the direction of bone—and then withdrawing and smelling the knife. The peculiar odour of good fresh meat is familiar to all, both in the raw and cooked state, and any departure from this would create suspicion.

The fat should have a firm and greasy feel; the normal faint yellow colour must not be excessive, although the fat of animals fed upon some oil-cakes acquires a very marked yellow hue. The fat deepens in colour with age. It should present no hæmorrhagic points.

Any lymphatic glands attached should be firm, smooth, slightly moist, and of a pale, greyish-brown appearance on section.

The pleura and peritoneum should be smooth, glistening, and transparent.

The marrow of the bones should be light red; that from the bones of the hind-quarters sets firmly within twenty-four hours, but that from the fore-quarters remains diffluent for a longer period.

The ash of the meat is alkaline, and consists almost entirely of phosphates and chlorides.

The Characters of Bad Meat

Bad flesh is frequently moist, sodden, flabby, and dropsical, and may be infected with parasites. It must be remembered, however, that the flesh of young animals is always pale and moist.

Some parts of the meat may feel softer than others—that is to say, there is not a uniform resistance to pressure, and occasionally there may be emphysematous crackling. The flesh of veal and lamb may be blown out artificially and the surface then smeared with fat, and thus an artificial plumpness is given to poor meat. Dishonest butchers may also rub melted fat over the flesh of diseased animals to give it a healthy and glossy appearance.

The fat is generally soft and flabby, or gelatinous; frequently highly coloured, or exhibiting small hæmorrhagic points.

Any attached lymphatic glands may be enlarged, softened, hyperæmic, ecchymosed, caseated, calcified, or suppurated. The marrow of the bones is discoloured (brownish) and sets badly.

A deep purple or dark tint suggests that the animal has not been killed and bled, but has died with the blood in it, and probably of some acute feverish condition or pulmonary complaint. A yellow or mahogany hue denotes bile-stained flesh.

Should the meat be very pale (" white flesh "), and the animal an adult one, fatty infiltration or degeneration, or fibroid infiltration, may be the cause. A magenta hue of the flesh points to some acute specific condition being present at the time of death.

Well-defined and dark-coloured areas full of blood are due to hypostatic congestion or post-mortem staining.

Pus may be seen lying between the muscle fibres, and boils or small abscesses may be present (as in anthrax, etc.).

There is frequently too great a proportion of bone to flesh, the animal having been greatly emaciated. The reaction of the juice (which may be dark or discoloured) may be alkaline or neutral.

The odour may be that of putrefaction or of a faint and sickly nature. The pleura and peritoneum may be wet or roughened, opaque, congested, or blood-stained.

Sometimes there is an odour of physic, as when, previous to death, odorous and volatile drugs (such as camphor, prussic acid, turpentine, creosote, chloroform, etc.) have been administered; or the animal may have fed upon odorous plants; or, subsequent to death, the carcass may have been hung in an atmosphere which is odorous from any cause (tobacco, carbolic acid, etc.).

It has been shown that no dangers to the meat would arise from the administration during life of medicaments such as arsenic, antimony (tartar emetic), or strychnine. The animal may in some cases take in poison by its food, by feeding upon such herbs as *bryony*, *meadow-saffron*, *rhus toxicodendron*, etc.

There are few changes which are so easy to detect as commencing putrefaction in fish; this is fortunate, inasmuch as decomposition sets in rapidly and appears to be more generally productive of poisonous symptoms than decomposing meat. The bright gills, the prominent eyes, the elastic resistance of the firmly adherent flesh, and the absence of any but the characteristic odour, are all evidence of freshness. The soft inelastic feel, the readiness with which the flesh can be detached from the bone, and the stale and unpleasant odour, furnish the chief clues—and the most reliable—of commencing decomposition. It has been

found possible to revive the greyish gills by artificial colouring agents, and to keep the eyes prominent by a small piece of stick, fixed transversely in the head, so that it presses the eye outwards on either side.

Greenness, iridescence, and sometimes luminosity, may be seen upon the surface of the flesh of decomposing fish. Stale fish float, while fresh fish sink in water.

In *putrefaction* of meat the flesh softens and tears readily; it becomes paler; the elastic resistance gradually diminishes and becomes less uniform—*i.e.*, some parts are softer than others; the characteristic odour is developed; the marrow softens and turns brownish; and the juices become alkaline in reaction, due to ammonia and substituted ammonias being formed by the action of schizomycetes. Later, the meat becomes of a greenish hue, and a glance then suffices to detect the presence of putrefaction. Occasionally meat becomes luminous, chiefly from the presence of *Bacillus phosphorescens*; but putrefaction eventually disperses this condition. Putrid meat may grow dull, dark moulds upon its surface.

As a test of putrefaction Eber recommends the use of a reagent composed of 1 part of hydrochloric acid, 3 parts of alcohol, and 1 part of ether. A few c.c. of this reagent are placed in a cylinder, which is then shaken so that the reagent applies itself to the inner surface of the cylinder. If a fragment of meat in the state of incipient putrefaction is introduced on the end of a wire, greyish or whitish fumes of ammonium chloride will generally appear.

The moulds which grow on the surface of meat are of numerous varieties—penicillium, mucor, phycomyces, verticillium, oöspora, etc. Red growths of *Bacillus prodigiosus* may also be associated with mould. There is little or no evidence that these growths or their products are injurious to health, although the meat is rendered unsightly and often unsaleable. Mould contamination is especially liable to occur when meat has been improperly handled or stored.

Certain diseases may cause characteristic appearances in the meat. When any such suspicion attaches itself to the sample of meat under examination, it is a great advantage to obtain a glance at the offal of the animal, and more especially to carefully inspect the liver, lungs, and lymphatics. The term "offal" includes the head, the feet, the skin, and all internal organs except the kidney; the remainder of the animal is termed the "carcass."

It is when there is evidence of parasitic attack that the flesh presents the most characteristic appearances.

Of those organisms which are commonly classified as "*animal parasites of flesh,*" some only are capable of infecting human beings when the flesh is eaten.

Harmless Animal Parasites of Flesh.

Coccidia oviformes (Leuckart) infest most animals (rarely man), and are chiefly found in the livers of rabbits, where they appear as small white nodules, which under the microscope are seen to contain clear ovoid bodies with either granular contents or egg-like structures known as sporoblasts. *Cœnurus cerebralis* forms hydatids, varying in size from a pea to a small walnut, in the brain and spinal cord of the ox and sheep. It is the cystic worm

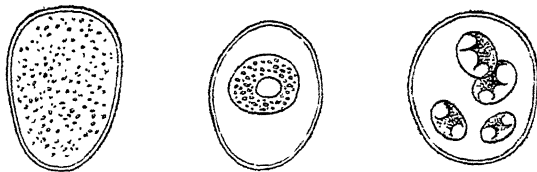



FIG. 59.—COCCIDIUM OVIFORME, SHOWING DEVELOPMENT OF SPOROBLASTS.

of *Tænia cœnurus* of the dog. *Cysticercus pisiformis* is found in the abdominal cavity and liver of the rabbit and hare; it is occasionally found in man, and the cysts are about the size of a pea. *C. tenuicollis* is found in the abdominal cavity of animals generally; it is the hydatid of the tape-worm, *T. marginata,* which inhabits the intestine of the dog; the cysts vary in size from a pea to a small orange, and do not invade the organs; the long thin neck is characteristic of the parasite. *C. serialis* is the immature form of a tape-worm affecting dogs; the cysts, varying in size from a hazel-nut to a pigeon's egg, are found under the skin and between the muscles. *Strongylus filaria* in the bronchial tubes of sheep, *S. micrurus* in the lungs of cattle, and *S. paradoxicus* in the lungs of the pig, are nematodes. There is another parasite found in the lungs of sheep, known as *Strongylus rufescens,* of which the eggs or embryos are deposited in the lung substance, forming little nodules which are usually of a greyish-yellow colour. This condition is found in adult animals, and is very common; it is called by many "pseudo-tubercu-

losis" of sheep. The lungs of at least 60 per cent. of all sheep slaughtered are affected by this parasite.

Certain nodular masses in frozen quarters of meat arriving from Australia have been found to contain the parasitic worm *Onchocerca Gibsoni*, which gives rise to a condition known as onchocerciasis.

Dr. Leiper found no evidence of vitality in the worm or in its embryo in the Australian beef arriving in this country, and it seems to be impossible for the parasite to develop in man from eating the affected meat. Apart, however, from this danger, the meat itself should be classed as unsound. The condition is most marked in the fore-quarters, where it is more or less confined to the region of the flank and brisket, and this has to be cut off of all Australian carcasses exported to this country.

DANGEROUS ANIMAL PARASITES OF FLESH.

Cysticerci.—The cysticerci, or "bladder-worms," cause the condition known as "measles" in the pig, ox, and sheep. *Cysticerci cellulosæ* are the bladder-worms which form a stage in the development of *Tænia solium*. In *the flesh of the pig*, and rarely in that of dogs, monkeys, or man, a number of small oval or round cysts are seen, occupying a position between the muscle fibres, and commonly of the size of a small pea—though they have been found as small as $\frac{1}{25}$ inch, and as large as $\frac{3}{4}$ inch, in length. They are surrounded by a pale. milky-looking fluid, and the cyst wall shows a white spot (generally central) upon its surface. The affected flesh is pale, soft, unduly moist and flabby, and has a smooth, slippery feel. The flesh does not set well, and quickly decomposes. Sometimes there is some degree of calcification of the capsule, and the result is that when sections are cut a grating sensation is experienced.

The bladders should be incised with a sharp knife, and the worm examined by a powerful hand-lens, when at one extremity will be found the blunt square head provided with a sucker at each "angle," and a fringe of about twenty-eight hooklets placed more centrally. These latter are very characteristic, and must always be found before a definite diagnosis is ventured upon.

Those cysts that are dried up and indistinct can be made visible by soaking in weak acetic acid. Ostertag attaches great diagnostic importance to the rounded or oval calcareous cor-

puscles which are so generally embedded in the tissue of the head, but which disappear on the addition of acetic acid. The liver and the muscles of the shoulders, intercostals, and loins are chiefly affected.

A staining test will generally suffice to determine if the cysticerci are alive or dead. If dead, the whole bladder-worm readily stains with carmine; if alive, the head at least resists the stain.

Cysticercus bovis, or "beef-measles," chiefly affects the calf, and is never found in man. It is somewhat smaller than *C. cellulosæ*, and possesses a flat head with no hooklets, but merely suckers, around which there is frequently a considerable deposit of pigment; and on the surface of the head there is a pit-like

FIG. 60.—HEAD OF TÆNIA SOLIUM. (OBJ. ½ INCH.)

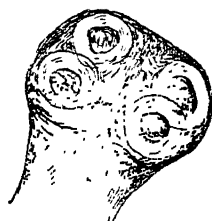



FIG 61.—HEAD OF TÆNIA MEDIOCANELLATA. (OBJ. ½ INCH.)

depression ("frontal suction cup"). It develops into the adult tape-worm called *Tænia mediocanellata*, or *T. saginata*, which is longer than *T. solium*.

Fish are subject to parasitic attack, and notably is this the case with the cod, in which many parasites have been found. Cooking effectually destroys them, for in the case of fish the flesh is not palatable unless the cooking is thorough.

Bothriocephalus latus, a tape-worm which is almost limited to certain parts of the continent of Europe, is even larger than *Tænia mediocanellata*, and has a club-shaped head, not armed with rostellum or hooklets, but possessing two deeply grooved longitudinal suckers, one on each side. The eggs are oval and comparatively large, with a characteristic operculum. Man is infected through eating imperfectly cooked fish, especially the pike, perch, and several members of the salmon family. There is no cysticercus form.

T. echinococcus is the small tape-worm, of three or four seg-

ments, which is commonly found in the dog. The encysted stage ("hydatids") is most generally found in the lungs and liver of oxen, sheep, and swine, but also (more especially in Iceland) in man. The hydatids consist of thin pale vesicles, floating in a clear liquid, and the whole is encysted in a tough capsule. The inner lining of the capsule consists of ciliated epithelium, and inside of the cyst wall there are generally many so-called "brood capsules" (Fig. 62). The cysts vary in size from a pin's head to that of a large orange. They may exist in such numbers in the liver that they replace the greater part of the entire tissue of that organ.

The condition is diagnosed with certainty by the microscope, either by the discovery of the characteristic heads or detached hooklets in the clear liquid of the cyst, valuable corroborative evidence being furnished by the fact that the liquid is quite

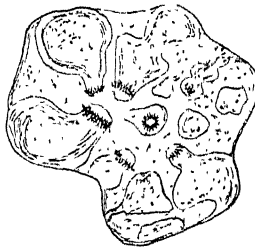



FIG. 62.—BROOD CAPSULE OF AN ECHINOCOCCUS.

free from albumin, and, in consequence, does not coagulate on boiling.

Tænia nana is the smallest human tape-worm (12 to 20 millimetres in length), and is not uncommon in Italy. The head contains four suckers, and a rostellum carrying twenty-two to twenty-four hooklets. It differs from *T. solium* in being very much smaller, and the rostellum of the latter carries a double row of hooklets, twenty-eight in number.

T. cucumerina is a little larger than *T. nana*; it occurs in man, especially in Norway and Sweden, but it is most common in the dog. The head contains four suckers, and three or four rows of hooklets (sixty in all) are disposed round the rostellum.

Among the *Cestoda* monstrosities may sometimes be observed, with abnormalities as to the number of suckers and hooklets.

Trichina spiralis.—This parasite has been found in the flesh of many different animals (pigs, pigeons, eels, etc.), but most

commonly by far in that of pigs; oxen and sheep do not suffer from attack by these nematodes. The disease is often seen in Germany, but rarely in England.

The shape of the minute worms is nearly that of a typical nematode—*i.e.*, a slender rounded body tapers gradually at either end; the extremity which constitutes the head goes to a long slender point, which presents a small central orifice, the mouth. The other extremity, the tail, ends more bluntly. The worm possesses a distinct alimentary canal, and even rudimentary sexual organs are present. In the female a uterus is discernible, which will frequently be seen to be full of minute free embryos curved upon themselves; these latter have been observed to become extruded from the vagina, and subsequently to move

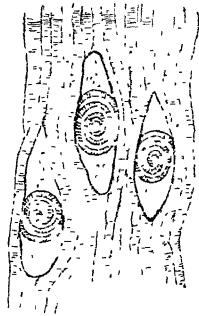



FIG. 63.—TRICHINA SPIRALIS, ENCYSTED IN MUSCLE. (× ABOUT 50 DIAMETERS.)

sluggishly about the field of the microscope. The male worm is much smaller than the female, and is only about $\frac{1}{16}$ inch long when mature; the length of the latter reaches $\frac{1}{8}$ inch. The long slender head and blunt tail are two characteristics which serve to distinguish these worms from parasites which otherwise resemble them, such as *Dracunculus* and *Filaria sanguinis hominis*.

The small worms are mostly coiled up in cysts, so disposed that their longest diameter is in a line with the muscular fibres, and a drop of acid will stimulate them to transient movements if they are alive. These cysts lie between the muscle fibrillæ, and their walls are sometimes partially or completely calcified, so as to give a grating sensation when the finger is passed over a section of the flesh. This calcareous deposit serves to shield the parasites from the destructive consequences of salting, and maybe even of cooking. There may be from one to three

trichinæ in a cyst. Frequently 25 per cent. of these parasites are thus encysted in the diaphragm, and therefore, when possible, a piece of this muscle should be procured; the back muscles, on the other hand, are the least attacked.

Either a section may be made of the muscle, or it may be teased out with needles; and in the case of a long muscle, a point near its insertion should be selected, since this is a favourite site for encystment. The affected muscle is seen to be pale and œdematous, and if the worms are encapsuled, small, rounded (or more truly, lemon-shaped), whitish specks, averaging about the size of a very small pin's head, are visible to the naked eye. These can be made very distinct by means of a hand-lens; but a low power of the microscope should be employed in every case, when the most characteristic appearance will be got by making a thin longitudinal section of the affected muscle and immersing this in potassic hydrate solution of medium strength, which serves to make the muscle fibres transparent and leaves the worm exposed in its coiled condition within the capsule. The soaking should not be prolonged beyond a minute or two, or the worm itself will also be cleared up. Glycerine is a good mounting medium when a permanent specimen is desired. Sometimes a view of the worm is obscured owing to considerable calcareous deposit in and around the walls of the capsule; in these cases a drop of dilute hydrochloric acid, run under the cover-glass, will dissolve up the deposit; or an oil globule, or several, may partially obscure the worm, when a drop of ether, applied in a similar manner to the acid, will clear away the fat. There are generally oil globules at the poles of the capsule.

The parts which are most likely to be affected will easily be remembered if it is borne in mind that the worms migrate to their settlements from the gastro-intestinal tract, and chiefly from the commencement of the small intestine. The diaphragm, the liver, the intercostal and abdominal muscles, are necessarily the first encountered, and therefore suffer most; but in later stages of the infection there is scarcely a muscle which may not be affected.

There are small, semi-transparent bodies called "psorospermia," or "Rainey's capsules," which to the naked eye resemble trichinæ; but they consist of small dark oval or elliptical bodies, of greater length than encysted trichinæ. They are, moreover, made up of a thick membrane formed by small hair-

like fibres arranged in lines, which encloses small, kidney-shaped, granular cells closely adherent together; and the whole lies embedded in the muscle substance itself—*i.e.*, the sarcolemma. They are extremely common, and may exist in the flesh of most of the animals used for human consumption, and apparently when eaten they produce no ill-effects.

Several other obscure bodies, the nature and significance of which we are still more ignorant of, may exist in flesh, such as bodies somewhat resembling pus cells, and others forming minute concretions or tiny hard nodules. Interesting as these are pathologically, they are rare; and when present, even in numbers, do not appear to affect the wholesomeness of the meat to any degree.

Actinomyces.—The "ray fungus" (actinomyces), one of the "fission fungi," is now recognized as a parasite of commoner

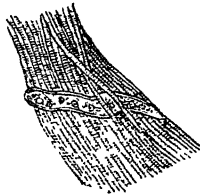



FIG. 64.—ONE OF RAINEY'S CAPSULES. (× 40).

occurrence in the ox than was once suspected; the difficulties which stood in the way of an earlier appreciation of this fact arose from the circumstance that both the ante- and post-mortem appearances of actinomycosis so closely simulate those of tuberculosis.

It has not yet been proved that the disease can be communicated by the flesh of animals (bovines) suffering from attack, and the vitality of the fungus when exposed to heat is very low.

The parasite almost entirely affects the tongue, the jaws (especially the lower one), the muscles of the cheek, and the lungs, where they may be detected by the naked eye as small dirty white specks, commonly about the size of a barley grain, but varying from the tiniest speck to $\frac{1}{3}$ inch in diameter. On section, the centre of the nodule is seen to be softer and of a greenish-yellow colour, or, less frequently, the nodule is firm and fibrous throughout. The condition is generally associated with considerable fibrous proliferation of affected parts. The parasites

assume, when encysted, a peculiar symmetrical appearance, due to the fact that they consist of small linear elements, thicker at one extremity than at the other, and are so arranged that their smaller extremities are all directed towards a central point; the stellate or rayed appearance thus created is sometimes remarkably regular and uniform. The tongue, when affected, is hard and swollen, and presents the flattened nodules chiefly upon its dorsal aspect. The size of these nodules may vary from $\frac{1}{6}$ inch to 2 inches, and the glands at the root of the tongue are also commonly infected.

Distoma hepaticum.—To examine for these parasitic trematodes, the liver should be taken and the bile ducts carefully exposed. In shape like little soles, they are of a pale-brown or slaty colour, and are provided at their broad extremity with two

FIG. 65.—DISTOMA HEPATICUM. (NATURAL SIZE.)

suckers, one at the anterior end and the other a little above the junction of the anterior and middle thirds of the median line. Their surfaces are beset with many little warty points, and they average in size from 1 to $1\frac{1}{2}$ inches in length and about $\frac{1}{2}$ inch in width. The bile ducts of the affected liver stand out on the surface ("pipy" liver). They are sometimes found encysted in the lungs of both sheep and cattle, when the cyst wall is usually calcareous and contains a chocolate-coloured fluid.

Frozen Meat.—Meat which has been frozen may be detected by expressing a drop of the meat-juice on to a glass slide, covering with a cover-glass, and examining by the microscope. The blood-corpuscles will be found to be much distorted in form, to have lost their pigment, and to be floating in a highly coloured serum; whereas the juice of fresh meat will show corpuscles of normal shape and colour, floating in a practically colourless serum.

Compared with fresh meat, frozen meat has usually a darker and more diffused red colour when thawed, owing to the hæmoglobin permeating the tissues; it is also somewhat softer.

Poisoning by Food.

There is no doubt that flesh in a very early stage of decomposition disagrees with many persons; and abundant evidence is not lacking that when an advanced state of putrefaction has been reached, violent gastro-intestinal irritation, followed by diarrhœa, vomiting, and toxic symptoms, may be induced.

Recorded cases of grave and fatal **food poisoning** have been very numerous, and minor disturbances of the gastro-intestinal tract are probably often due to small doses of poisons produced by bacteria. Often the offending substance appears, to all physical tests, to be quite good and wholesome; but the meat or jelly formed is sometimes observed to be softer and moister, and a slight peculiar odour has been noted. The poisonous food has most commonly been brawn, sausages, ham, pork, veal pie, rabbit pie, potted shrimps, tinned salmon, mackerel, mussels, and oysters; but many other varieties of food have been incriminated, such as cheese, ice-cream, canned goods, potatoes, etc. There is a strange absence of recorded instances where the flesh of sheep has been the offending food; but this is seldom used in the preparation of made foods.

In food which has become poisonous, a living micro-organism has produced an organic chemical poison, which may be a ptomaine, albumose, or toxine. The substance is the immediate cause of the morbid symptoms, and is probably produced by the action of the micro-organisms on the albuminous constituents of food. Both the products of specific micro-organisms in an infected food and those basic substances resulting from putrefactive micro-organisms ("ptomaines") may be fleeting, as regards their existence, since the micro-organism may be killed by its own products or by heat, or the chemical poison (from its unstable nature) may undergo decomposition; so that an infected food which may be poisonous at one time may fail to be poisonous at another. The micro-organisms may produce their peculiar chemical poisons from material affording them nourishment, which may be either outside the body of man or within it. Food-poisoning outbreaks are far more prevalent in the summer months.

In the majority of cases the symptoms of poisoning occur within twelve hours, when they are due to the ingestion of already formed poisons ("intoxication"); but in other cases the symp-

toms may be delayed for twelve to forty-eight hours, when they are probably due to a food "infection" by organisms which produce poisons after the food is taken into the human body. Generally there is a mixture of bacilli and toxines, and therefore variable incubation periods. In most cases the symptoms include considerable abdominal pain and tenderness, vomiting and diarrhœa, with tenesmus, headache, and marked depression or collapse. Other frequent symptoms include dilated pupils, rashes, and albuminuria. The temperature may be raised, but generally it is subnormal. Although the acute symptoms disappear after two or three days, marked prostration may persist for a much longer period.

The mortality among those affected by meat poisoning is often from 2 to 5 per cent., and except in cases where rapidly fatal results follow, a post-mortem examination usually discloses gastro-enteritis, and sometimes ulcers in the small and large intestines, with enlarged spleen and congested liver and kidneys.

The most frequent form of meat poisoning results from consuming the flesh of diseased animals, and is associated with the presence of *Bacillus enteritidis* (Gaertner and allied organisms) and the *paratyphoid bacillus;* but healthy carcasses may be infected in the slaughter-house by the knives used for the purpose of dressing many animals, and cooked or uncooked meat may be infected by rats, mice, flies, dust, ice, or the soiled hands of human "carriers." Ptomaine poisoning from eating putrefied meat, and usually associated with the presence of *B. proteus* and *B. coli*, is less common; and "botulismus," which results from the toxine of *B. botulinus*, is of extreme rarity in this country.

Ptomaine poisoning has most frequently resulted from eating meat in a cut-up condition (sausages, minced meat, etc.), and game; and especially meat which has been insufficiently cooked (for the thorough cooking of meat destroys *B. proteus* and its toxines).

Ptomaines are not the cause of extensive outbreaks of meat poisoning. The infection of food by the bacilli of the Gaertner group is the common cause of these. *B. suipestifer*, found in the pig's intestine, is a member of the Gaertner group, and is the bacillus of swine fever. This organism has been shown to be responsible for some outbreaks of poisoning following the consumption of pig's flesh.

The power of certain shellfish to create violent gastro-intestinal

disturbance and urticaria is well recognized; mussels, cockles, and oysters collected from near sewage outfalls have had virulent poisonous properties ascribed to them, and they may convey the infection of enteric fever. From mussels a poisonous ptomaine, mytilotoxine, has been isolated by Brieger. This exists chiefly in the hepatic organ of the mussel. Acute gastro-intestinal and profound nervous symptoms may follow the consumption of either the raw or cooked mussel. Perch, sturgeon, turbot, pike, crabs, shrimps, salmon, and sardines have all given rise to poisoning, either from the development of putrefaction toxins, or from bacterial infection or intoxication. Some fish normally contain a toxine poisonous to man, others develop such a poison only in the spawning season. The consumption of canned fish may give rise to symptoms akin to "botulismus."

Some ptomaines are highly poisonous, while many are inert. The majority of the known ptomaines contain only C, H, and N, and represent simple ammonia substitution compounds. The kind of ptomaine formed will depend upon the organism present, the nature of the food substance, the temperature, the stage of putrefaction, etc.

A large number of toxic bodies have been isolated and described, some of the more important being—

Methylguanidine ..	Poisonous.
Dihydrocollidine ..	Poisonous.
Neurine	Poisonous.
Choline	Poisonous.
Muscarine	Poisonous. Obtained from poisonous mushrooms and fish.
Gadinene	But little poisonous. Obtained from putrid fish.
Mytilotoxine	Poisonous. Obtained from mussels taken from positions liable to sewage pollution.
Tyrotoxicon	Poisonous. Obtained from cheese, cream, and ice-creams.

The *B. botulinus* is an anaerobic organism, and botulism generally results from the eating of sausages in thick skins (German sausages) and other food which has been hermetically stored or embedded in fat. The bacillus has been found in ham, tinned fish, lobsters, oysters, and cheese.

In botulismus the main symptoms are not gastro-intestinal, but nervous, such as dryness of mouth and throat, difficulty in swallowing, ptosis, double vision, convulsive muscular tremors, vertigo, constipation, retention of urine, disturbances of heart's action and of respiration; consciousness is unimpaired, and the combined symptoms, which generally appear in from twelve to twenty-four hours, though sometimes earlier, are akin to those of atropine poisoning (Dieudonné). Vomiting is not uncommon.

Vanilla flavouring in sauces or ice-cream has often given rise, within two hours, to vomiting, tenesmus, diarrhœa, and signs of collapse. The explanation appears to be that the vanillin, by its reducing action, favours the growth of anaerobic bacteria, the vanilla itself being harmless.

Potatoes may give rise to similar symptoms of poisoning, which are said to be due in some cases to a considerable increase of the trace of the poisonous substance "**solanin**" to be found in normal potatoes, but generally to bacterial decomposition by proteus bacilli (Dieudonné). It has been recommended that in order to guard against solanin poisoning, the peel and any sprouts should always be thoroughly removed.

The alkaloidal glucoside "solanin" decomposes in the human body into glucose and the alkaloid "solanidine," the poisonous and fatal effects of which have often been recorded. For its isolation Meyer's method may be used. Five hundred grammes of the pared potatoes are finely divided with a grater, and the liquid portion separated by pressing in a muslin cloth. After standing, the liquid is decanted from separated starch, which is washed with small quantities of water, the washings being added to the original liquid. Render alkaline with ammonia and evaporate almost to dryness; boil the residue with alcohol and filter. This treatment with alcohol is twice repeated. The pressed solid portion is also boiled with alcohol in two successive portions and filtered. Evaporate the united alcoholic solutions to a small bulk, allow to stand overnight, filter. The filtrate is evaporated to dryness and digested overnight with 250 c.c. of water containing about 3 c.c. concentrated H_2SO_4. After filtration and washing, the solanin is precipitated by an excess of ammonia and warmed to 50° C. Filter, wash, and dissolve the solanin by pouring hot alcohol through the paper, receiving same in a weighed crystallizing dish. Evaporate the solvent,

dry, and weigh. The normal solanin content is about 0·06 to 0·08 part per 1,000 parts of potatoes.

Cheese, milk, cream, butter, and ice-creams, etc., may all rarely contain **tyrotoxicon,** which develops under those circumstances most conducive to fermentative changes generally—viz., warmth and moisture, impure and confined air, and deficient light. It is a diazo-benzene-butyrate, and is found to occur under conditions of improper storage in the various food articles above mentioned; and when the ingestion of any of these articles has given rise to serious consequences, then a search should be instituted for this most powerful poison. The symptoms it creates commonly pass off within a few hours, but occasionally serious consequences have arisen, such as the development of symptoms akin to atropine poisoning, which may be followed by fatal collapse. The physical characters of the article are not necessarily altered in any way, but acidity is always marked, and where this is normally present (as in cheese) it is invariably increased.

The *method of examination* in the case of milk is as follows:

1. The filtrate from the milk is first rendered distinctly alkaline by means of sodium carbonate; then an equal bulk of pure ether is added, and the whole well shaken up in a separator.

2. The mixture is next allowed to stand until all the ether has separated into a layer upon the surface.

3. This ethereal layer is then decanted on to a saucer, where it is left until the ether has spontaneously evaporated and a comparatively dry residue remains.

4. The residue is carefully dissolved in a little pure water and then filtered to free it from fat.

5. The filtrate is next well shaken with an equal bulk of pure ether, and the ethereal layer, having separated, is removed and allowed to again evaporate spontaneously.

6. The residue left upon the saucer will then contain any tyrotoxicon which may have been originally present, sufficiently pure to respond to the following test: on the addition of a few drops of a mixture of equal parts of pure carbolic and sulphuric acids, if the poison be present, a reddish colour appears.

In the case of cheese and butter, these are first thoroughly worked up (triturated) with water, and the filtered extract is then treated in the manner indicated above. It is probable that cheese poisoning is usually due to toxines produced by bacteria.

In order to investigate cases of alleged food poisoning a considerable amount of the suspected material is necessary. Perishable articles should be placed in an ice-box. The amount generally submitted for examination is often too small to admit of a thorough bacteriological and chemical examination. The bacteriological evidence will be furnished by the morphological characters of the organisms found in the food, or the sufferer's vomit, fæces, etc., their pathogenicity, and serum reactions. With respect to the chemical examination, that will be concerned more particularly with the nature and amount (if present) of chemical preservative, metallic contamination, or colouring agent; little is to be gained by testing for the presence and attempting to define the nature of "ptomaines."

Rats and mice should be fed with portions of the sample, while other animals—*e.g.*, guinea-pigs—should be inoculated subcutaneously and intraperitoneally from cultures and also from the broth emulsions of the food. If any of the animals die, a complete post-mortem examination should be made.

It is also advisable to cut sections and otherwise microscopically examine the meat to see if the bacilli are chiefly on the surface, and also if the meat-fibres are from apparently healthy animals.

An autopsy should be made in all cases of death from food poisoning of this nature, and cultures made from the different organs (spleen, mesenteric glands, intestines, etc.).

As already mentioned, the agglutinative properties developed in the blood of suspected cases (sufferers or carriers) should be made use of for diagnostic purposes.

The alkaloidal products of putrefaction are separable by their relative solubility in alcohol, ether, chloroform, etc.

The following method (after Stas) may be adopted:

The finely mixed material is drenched with 90 per cent. alcohol, tartaric acid is next added until the liquid is definitely acid (if the fluid is already acid this is not necessary), and the mixture is then allowed to digest for several hours at 70° to 75° C. After cooling, the alcoholic liquid is removed (the last portions by pressure) and filtered. This operation is repeated several times, and the united filtrates are evaporated *in vacuo* at 35° C. to a small bulk. The liquid is then filtered through a wet filter to remove fatty matter; powdered glass is now added to the filtrate, and the mixture evaporated nearly to dryness over

sulphuric acid in a vacuum. The residuum is next digested in pure alcohol for twenty-four hours, and again evaporated (at 35° C. *in vacuo*) to dryness. This residue is dissolved in a little water made alkaline with sodium bicarbonate, and the solution is then well shaken with 4 volumes of pure ether. Finally, the ethereal solution is decanted, evaporated to dryness, and the alkaloid is left behind.

The **extract** which may thus be obtained may be dissolved and tested with various reagents in order to ascertain its particular nature; or it may be given to a lower animal and its effect noted, if it is only necessary to learn whether the original material was harmful or not. The best animals for such experimental purposes are mice.

A quantitative and qualitative bacteriological examination of **oysters** and other shellfish is often very desirable in the interests of public health, to see how far they are free from excretal and sewage pollution bacteria. The most thorough method (Houston) for the bacteriological examination of oysters is briefly as follows:

1. The outsides of the oyster-shells are well scrubbed with soap and water, and cleaned as thoroughly as possible in running tap-water, finally with sterile water.

2. Hands of the investigator are thoroughly cleaned, washed in 1 in 1,000 corrosive sublimate solution, and finally with sterile water.

3. Oysters opened by a sterile knife held in position by a sterile cloth, and with concave shell underneath. Great care must be taken to avoid any loss of the liquor. The liquor in the shell is poured into a sterile 1,000 c.c. cylinder, and the oyster and oyster liquor are added after the oyster has been cut into small pieces by sterile scissors.

4. Ten oysters are to be treated as above in each experiment.

5. The volume of oyster + oyster liquor is read off, and usually varies between 80 and 120 c.c. For qualitative work 100 c.c. may therefore be taken as a fair average of the total shell contents of ten oysters.

Sterile water is then poured into the cylinder up to the 1,000 c.c. mark, and the whole well stirred with a sterile rod. Each 100 c.c. of this liquor may be considered to contain the bacteria in one oyster.

6. Various amounts and fractions of this liquor are used for the examination for *B. coli, B. enteritidis sporogenes*, and for streptococci.

For the *B. enteritidis sporogenes* examinations Houston used 10, 1, 0·1, 0·01, 0·001 c.c., and for *B. coli* these dilutions, and in addition 100 c.c. and 0·0001 c.c. He made these primary cultures in triplicate, and required at least two out of the three to be positive for a preliminary numerical diagnosis.

Cockles and mussels may be examined by a very similar procedure.

Ice-Cream.—There is a considerable probability, and some evidence, that ice-cream may be a means of spreading disease, particularly typhoid fever. In investigating such a possibility the bacteriological examination of ice-cream is of value.

The ice-cream should be collected in a sterile vessel—*e.g.*, a wide-mouth sterile bottle with glass stopper—and packed in ice if it cannot be examined at once.

To examine, melt the ice-cream by placing for fifteen to twenty minutes in the 22° C. incubator, then treat as a milk sample.

Edible and Poisonous Fungi.—With a view to enable residents in the country to distinguish between the poisonous and edible kinds of fungi, and to utilize to a greater extent those varieties which are useful as food, the Board of Agriculture and Fisheries has published a small book of illustrations of those species which are more commonly found in Great Britain, together with brief descriptions of them. It is pointed out in this publication that, contrary to popular belief, the poisonous kinds of fungi are comparatively few in number, while there are, on the other hand, some fifty species of edible fungi which may safely be eaten. In order to recognize with certainty these different kinds, it is necessary to know those special features possessed by each species which separate it from all others. The rule-of-thumb signs for discriminating between edible and poisonous fungi are valueless, and no reliance should be placed on the presence of a skin that is readily peeled off as an indication of an edible fungus, or on the statements that a silver spoon placed in contact with poisonous kinds becomes tarnished, and that all fungi growing on wood are poisonous.

In England and France most of the cases of fungus poisoning which have been reported are due to *Amanita phalloides*. Muscarin is the toxine to which mushroom poisoning has been com-

monly ascribed, but the poison of this fungus is probably a toxalbumin. *Amanita phalloides* is white beneath the cap, with a yellowish-white or greenish-white, shining top; and the stem, which is white and smooth, is bulbous, and is clothed at its upper part with an expanded pendant ring. It usually occurs in woods, and it rarely, if ever, grows far away from trees, having a preference to the proximity of the oak variety of tree. The fungus peels almost as well as the common mushroom. Another less common offender is *Lactaria torminosa*.

HORSE-FLESH.

In horse-flesh the meat is darker and more brownish; it is coarser (the muscular fasciculi being broader) than in ox-flesh; the odour of the fresh meat is different, and after the lapse of a day or two, as the flesh dries, it develops a peculiar faint odour, a bluish sheen, and imparts a soapy feeling to the fingers. The fat is more yellow and soft, and possesses a sickly taste; and, in consequence, it is sometimes removed and replaced by ox-fat, which is skewered on to the meat. If the bones have not been removed, they will afford an additional clue, inasmuch as they are larger and their extremities (tuberosities, etc., for the attachment of muscles and ligaments) are larger and more marked; there are, in addition, some anatomical differences in the construction of the horse's skeleton. In cattle the breast-bone is broad and flattened, while in the horse the front portion is keel-shaped. The ribs in cattle are flatter and broader in the middle and lower thirds than in the horse.

Horse-flesh is richer in glycogen than ordinary meat, and as this remains unchanged for a long time it is taken advantage of in the following test:

An infusion of the suspected meat is prepared by boiling 100 grammes of it, in a finely minced condition, with ½ litre of water, for nearly one hour; the broth is then concentrated to about 100 c.c., and is mixed, when cold, with dilute nitric acid in the proportion of 5 c.c. to 100 c.c. of the broth; it is then filtered and tested with hot (freshly prepared) saturated solution of iodine, gently let down upon it so as not to mix the liquids. If the substance is horse-flesh, a *marked* reddish or brownish-violet ring appears at the junction of the two liquids. If a deep violet colour results, starch is present, as may be the case in sausage-

meat. The iodine solution should consist of iodine, 1 gramme; iodide of potassium, 2 grammes; water, 100 c.c.; and it must be carefully added, since a slight excess changes the colour to reddish-brown. Glycogen is present in the livers of cattle and in meat extract, and the dextrines derived from starch in sausages give a similar reaction; therefore it is only when the amount found is considerable that the glycogen reaction can be taken as certain evidence of horse-flesh in sausages. Moreover, in old sausages containing horse-flesh the glycogen is liable to undergo decomposition, and then no reaction is obtained. In smoked horse-flesh the glycogen is destroyed.

Certain organs of the horse are occasionally sold as the corresponding organs of the ox. The tongue of the horse is broad and rounded at its free end, instead of pointed, as in the ox; and it is smooth at the base, where the tongue of the ox is rough with prominent papillæ. If the hyoid bone is attached, it is found to be made up of five parts, whereas that of the ox consists of nine. The epiglottis is, moreover, smaller and more pointed in the horse. The liver, whether of the ox or sheep, consists of one very large lobe, and another relatively small one; in the horse there are three large and distinct lobes, and a fourth which is very small, and there is no gall-bladder.

The kidney of the horse is more heart-shaped, and cannot be mistaken for the long lobulated kidney of the ox. The heart of the horse differs from that of the ox in having less fat at its base and in being less conical; it is, moreover, darker and softer. There is a bone in the heart of the ox (os cardis).

Sausages.

These are made of the chopped flesh or internal organs of various animals, mixed with condiments, flour, bread, or potato meal, and filled into clean gut or parchment; the sausages are then generally boiled, smoked, or scalded. Saltpetre is sometimes added to furnish a good red colour to the meat, and often colouring agents (carmine, cochineal, or aniline) and preservatives are added. The colouring matter can generally be extracted by warming for several hours with a mixture of equal parts of glycerine and water. Boric acid is often used as a preservative. It is certain that since boric acid prevents objective decomposition, such as is detectable by odour, it permits of the use of

stale meat and meat in the early stages of decomposition for the making of sausages. Dr. C. A. MacFadden, in a Report to the Local Government Board (1908) expresses the view that if boron preparations are permitted, 0·25 per cent. (17·5 grains per pound) of boric acid should be not exceeded, and that even then such addition should be made known to the purchaser.

The use of antiseptics in sausages is to be discouraged. Even the boric acid (about 20 grains to the pound), which is most frequently employed, may be injurious to those with weak stomachs or kidneys. Meat sausages (pork and otherwise) can be made, sold, and consumed without any such addition, if good fresh material is employed, for people do not purchase sausages to store in the house; and the dried "German" sausages can be sufficiently preserved by other means.

While the amounts of boric acid usually employed will not enable the use of meat which has reached a stage of marked putrefaction, they permit of the use of stale material in a state of incipient decomposition, and while they may reduce the danger from putrefactive toxines, they do not materially reduce the risks of poisoning from organisms of the Gaertner group or from *Bacillus botulinus*. Owing to its cheapness (beef sausages are generally sold at about 1s. a pound), the poorer people consume a considerable quantity of sausage, ¾ pound of which could be eaten at a meal by an adult. That the use of chemical antiseptics is unnecessary is demonstrated by the large number of makers who never employ them. The writer has satisfied himself, by experiments, that if the meat is sound and fresh at the time it is put into a sausage it will keep in hot weather for forty-eight hours, and that as little as 12 grains of boric acid to the pound will enable the sausage to be kept in such weather for four days; so that even if antiseptics are permitted, and it is necessary to keep fresh sausages for four days, there is no case for the employment of the 20 to 30 grains of boric acid to the pound which is sometimes found to be present.

It is not a difficult matter to detect early decomposition in *sausages*; the alteration in the odour will sometimes suffice; for if a little of the sausage is boiled with water and some freshly prepared lime-water is added, good meat yields only a faint ammoniacal odour, whereas bad meat will give off a peculiarly offensive ammoniacal odour. Putrefaction generally commences

in the middle of the sausage, when a dirty greyish-green colour is often to be noted.

There is great variation in the composition of sausages; but commonly the moisture amounts to 50 to 65 per cent., and of the dried material the bread or meal ranges from 6 to 30 per cent., and the flesh from 50 to 80 per cent.

The skins of sausages have been known to contain mineral poisons, but this is very rare.

EGGS.

The best tests for bad and stale eggs are the following:

1. Fresh eggs are most *translucent* towards their centres if held vertically against the light; stale eggs are most translucent at their upper extremities.

2. If 2 ounces of salt are dissolved in a pint of water, fresh eggs when placed in the solution sink, and stale ones float.

Opaque spots are generally due to moulds that have gained access through a crack or cracks in the shell, or to embryos. Eggs generally contain bacteria and sometimes parasitic worms, but there are no recorded instances of human infection.

Toxic poison has been separated from decomposing eggs.

MEAT PREPARATIONS.

Many **meat extracts** are now upon the market, the tendency being for the public to over-estimate their food value. They consist of the extractives of meat, and not of the meat itself; and they act as stimulants and regulators of digestion rather than as true foods capable of providing the necessary amount of nitrogenous material for the needs of the body.

A meat extract should consist of a golden-brown sticky substance, with a pleasant meaty odour. It should never be hard, and should attract moisture strongly from the air. The reaction should be slightly acid. The usual method of preparation consists in heating raw meat, which has been finely divided, with a little water under pressure. The extract thus made is filtered and evaporated *in vacuo*. It is essential that a temperature below 75° C. be employed if all gelatine is to be excluded (Beveridge). The extract thus made contains the flesh bases or extractives and mineral matters of the meat, and is free from albumin, meat fibre, gelatine, and fat; but in some of the meat extracts on the market these substances and also

vegetables are subsequently added in order to give the extract a certain food value. Meat juices are prepared in the cold by subjecting finely divided meat to strong pressure, and ultimately concentrating by evaporation *in vacuo*. They contain the soluble proteins of meat.

Protein matter constitutes the bulk of meat extract, but it varies greatly in amount in different samples. The total nitrogen, calculated as protein, should not be less than 55 per cent., of which not less than 25 per cent. must be insoluble in alcohol. Protein soluble in alcohol is presumed to consist of half meat bases and half meat extract, but from a practical point of view these distinctions are unnecessary in routine examinations. Water should not exceed 25 per cent. and the ash 20 per cent.; the former varies from 14 to 25 per cent., and the latter from 14 to 33 per cent., in samples of the usual brands of meat extracts.

Meat Essences.—A meat essence is a more liquid extract, containing more water, but has the same colour, odour, and reaction. Protein matter should not be less than 9 per cent., of which not less than 7 per cent. should be insoluble in alcohol. Water should not exceed 90 per cent., nor the mineral ash 1·5 per cent.

The analysis of a meat essence is similar to that of a meat extract, about ten times as much of the substance being taken for each examination.

On account of the large amount of water present, frothing of the contents of the Kjeldahl flask is apt to be troublesome, and unless the first stage of the process is carefully watched the flask contents may be ejected.

The gelatine in **table jellies** usually amounts to from 13 to 17 per cent., so that what nutrient value they possess is derived mainly from the sugar, which amounts to from 50 to 80 per cent. W. W. O. Beveridge gives the following method for obtaining a closely approximate estimation of gelatine:

Twenty-five grammes of the material containing gelatine are dissolved in hot water, and filtered if necessary to remove any insoluble matter. The solution is evaporated to a thick syrup on the water-bath in a platinum capsule, then removed and cooled; 5 c.c. of a 10 per cent. solution of formaldehyde is then added, which renders the gelatine insoluble. Other proteins must not be present. The soluble matters, such as sugar, etc., are dissolved out by means of boiling water, when the gelatine remaining behind can be dried and weighed.

CHAPTER IX

ALCOHOLIC BEVERAGES

THE law allows a margin of 2 per cent. of proof-spirit before regarding a beverage as an alcoholic fluid.

The estimation of **alcohol** in alcoholic beverages may be made as follows:

Three hundred c.c. of the beverage (generally diluted) is placed in a retort and boiled gently until about 200 c.c. have distilled over into a flask. The distillate is next made up to the original bulk of 300 c.c. with distilled water, and the specific gravity is taken in a S.G. bottle (*vide* p. 7), when the temperature has cooled to about 15° C. If this is 1,000 the fluid is free from alcohol, and the amount of alcohol which has distilled over will be great in proportion to the extent to which the S.G. falls below 1,000, since pure absolute alcohol at 15° C. has a S.G. of 793·8. To find the percentage amount of alcohol from the S.G. of the distillate, the alcohol table on pp. 318 and 319, in which these data are arranged side by side, may be consulted.

In estimating alcohol in spirit, take 100 c.c. of the spirit, and dilute with 200 c.c. of distilled water. Before commencing the distillation of beer, it should be well shaken to expel as much carbonic acid as possible, passed through a coarse filter-paper, diluted with an equal volume of water, and rendered alkaline with caustic soda; and as a further precaution against the beer frothing over, a small flame only should be applied to the flask and a little tannin powder may be added.

An estimation may be made without distillation in the following manner: Ascertain the specific gravity at 15° C. of the original liquid, then take 300 c.c. and boil down to about 100 c.c., thus driving off the alcohol; make up to the original bulk with distilled water, and again take the S.G. at 15° C. The difference between the first and second gravities deducted from 1,000

(the gravity of water) gives the S.G. of the alcohol evaporated. If, for instance, the first S.G. of a sample of beer is 1,009 and the second S.G. is 1,018, then 1,018 − 1,009 = 9, and 1,000 − 9 = 991. A reference to the table on p. 318 will show that a liquid with a S.G. of 991 (or 0·991, as there expressed) contains 6·55 per cent. of alcohol by volume.

SPIRITS.

Spirits constitute the distillates from various liquids containing alcohol derived from grain.

The expressions "over proof," "proof," and "under proof" are commonly employed to denote the amount of alcohol in spirits. The above terms had their origin in a former practice of pouring the spirit over gunpowder, and applying a light to it. If the spirit burned without igniting the powder, owing to the large admixture of water, it was "under proof"; and the weakest spirit capable of firing the powder was called "proof." Such a spirit was stronger than the present "proof-spirit," for by "proof-spirit" is now implied a mixture of 57·06 per cent. by volume, or 49·24 per cent. by weight, of pure absolute alcohol in water, with a S.G. of 0·9198 at 15° C.; and solutions weaker or stronger than this are "under" or "over" proof.

The proportions of alcohol in alcoholic fluids may be stated as either a percentage of alcohol by weight, a percentage of alcohol by volume, or a percentage of proof-spirit.

The percentage of alcohol by weight may be obtained by multiplying the percentage by volume by 0·7938 and dividing the product by the specific gravity; and, conversely, the percentage of alcohol by volume may be obtained by multiplying the percentage by weight by the specific gravity, and dividing the product by 0·7938.

The percentage of proof-spirit may be obtained by multiplying the percentage of alcohol by volume by 1·75. A percentage of proof-spirit is always expressed by volume.

The Sale of Food and Drugs Acts fix the following low limits of alcohol in "spirits." Brandy, whisky, and rum may be 25° "under proof," corresponding to 75 per cent. of proof-spirit—*i.e.*, the S.G. may be as high as 0·9474—and there may be only 42·8 per cent., by volume, of absolute alcohol.

Gin may be 35° "under proof"—*i.e.*, may only contain 37·1 per cent. by volume of absolute alcohol; and the S.G. may

be as high as 0·9563, corresponding to 65 per cent. of proof-spirit.

Suppose a sample of whisky is 44° under proof; it therefore contains $100-44=56$ per cent. of proof-spirit. What percentage of spirit of 25° under proof does it contain? A spirit of 25° under proof contains 75 per cent. of proof-spirit. Therefore the whisky contains $\frac{56\times 100}{75}=74\cdot6$ per cent. of spirit of required strength and 25·4 per cent. of added water.

The percentage amount by weight of absolute alcohol generally present in spirits=32 to 50; wines=8 to 18 (about 10 per cent. in clarets); strong ales and porter=5 to 8; bottled cider=3 to 7; small beer=2 to 3. Lager beer contains more dextrine than English beer, and usually less alcohol.

SHORT ALCOHOL TABLE.

Specific Gravity at 15° C.	Per Cent. of Alcohol by Volume.	Per Cent. under Proof.	Specific Gravity at 15° C.	Per Cent. of Alcohol by Volume.	Per Cent. under Proof.	Specific Gravity at 15° C.	Per Cent. of Alcohol by Volume.	Per Cent. under Proof.
1·000	0·00	100·00	0·972	24·08	57·80	0·944	44·79	21·50
0·999	0·66	98·84	0·971	25·07	56·06	0·943	45·41	20·43
0·998	1·34	97·66	0·970	26·04	54·37	0·942	46·02	19·36
0·997	2·12	96·29	0·969	26·95	52·77	0·941	46.59	18·36
0·996	2·86	95·00	0·968	27·86	51·18	0·940	47·13	17·40
0·995	3·55	93·78	0·967	28·77	49·60	0·939	47·67	16·46
0·994	4·27	92·50	0·966	29·67	48·00	0·938	48·21	15·50
0·993	5·00	91·23	0·965	30·57	46·44	0·937	48·75	14·57
0·992	5·78	89·87	0·964	31·40	44·97	0·936	49·29	13·63
0·991	6·55	88·50	0·963	32·19	43·60	0·935	49·81	12·70
0·990	7·32	87·16	0·962	32·98	42·20	0·934	50·31	11·82
0·989	8·18	85·65	0·961	33·81	40·74	0·933	50·82	10·94
0·988	9·04	84·15	0·960	34·54	39·47	0·932	51·32	10·05
0·987	9·86	82·70	0·959	35·28	38·18	0·931	51·82	9·20
0·986	10·73	81·20	0·958	36·04	36·83	0·930	52·29	8·36
0·985	11·61	79·65	0·957	36·70	35·68	0·929	52·77	7·52
0·984	12·49	78·10	0·956	37·34	34·57	0·928	53·24	6·70
0·983	13·43	76·46	0·955	38·04	33·32	0·927	53·72	5·86
0·982	14·37	74·82	0·954	38·75	32·08	0·926	54·19	5·03
0·981	15·30	73·18	0·953	39·47	30·84	0·925	54·66	4·20
0·980	16·24	71·54	0·952	40·14	29·66	0·924	55·13	3·38
0·979	17·17	69·90	0·951	40·79	28·52	0·923	55·60	2·56
0·978	18·25	68·00	0·950	41·32	27·60	0·922	56·07	1·74
0·977	19·28	66·20	0·949	41·84	26·67	0·921	56·54	0·92
0·976	20·24	64·53	0·948	42·40	25·70	0·920	56·98	0·14
0·975	21·19	62·87	0·947	42·95	24·74	0·9198	57·06	-Proof
0·974	22·18	61·13	0·946	43·56	23·66			
0·973	23·10	59·52	0·945	44·18	22·58			

SHORT ALCOHOL TABLE—*continued.*

Specific Gravity at 15° C.	Per Cent. of Alcohol by Volume.	Per Cent. over Proof.	Specific Gravity at 15° C.	Per Cent. of Alcohol by Volume.	Per Cent. over Proof.	Specific Gravity at 15° C.	Per Cent. of Alcohol by Volume.	Per Cent. over Proof.
0·919	57·45	0·68	0·876	75·45	32·23	0·833	90·29	58·23
0·918	57·92	1·51	0·875	75·83	32·89	0·832	90·58	58·74
0·917	58·36	2·28	0·874	76·20	33·54	0·831	90·88	59·26
0·916	58·80	3·05	0·873	76·57	34·19	0·830	91·17	59·77
0·915	59·22	3·78	0·872	76·94	34·84	0·829	91·46	60·28
0·914	59·63	4·50	0·871	77·29	35·45	0·828	91·75	60·79
0·913	60·07	5·27	0·870	77·64	36·07	0·827	92·05	61·32
0·912	60·52	6·07	0·869	78·00	36·69	0·826	92·36	61·86
0·911	60·97	6·86	0·868	78·36	37·33	0·825	92·66	62·38
0·910	61·40	7·61	0·867	78·73	37·98	0·824	92·94	62·88
0·909	61·84	8·36	0·866	79·12	38·65	0·823	93·23	63·38
0·908	62·31	9·20	0·865	79·50	39·32	0·822	93·49	63·84
0·907	62·79	10·03	0·864	79·86	39·96	0·821	93·75	64·30
0·906	63·24	10·84	0·863	80·22	40·60	0·820	94·00	64·74
0·905	63·69	11·64	0·862	80·60	41·26	0·819	94·26	65·18
0·904	64·14	12·41	0·861	81·00	41·96	0·818	94·51	65·62
0·903	64·58	13·18	0·860	81·40	42·66	0·817	94·76	66·07
0·902	65·01	13·92	0·859	81·80	43·35	0·816	95·03	66·53
0·901	65·41	14·62	0·858	82·19	44·04	0·815	95·29	67·00
0·900	65·81	15·33	0·857	82·54	44·66	0·814	95·55	67·46
0·899	66·25	16·11	0·856	82·90	45·28	0·813	95·82	67·92
0·898	66·69	16·88	0·855	83·25	45·90	0·812	96·08	68·38
0·897	67·11	17·61	0·854	83·60	46·51	0·811	96·32	68·80
0·896	67·53	18·34	0·853	83·94	47·11	0·810	96·55	69·20
0·895	67·93	19·05	0·852	84·27	47·70	0·809	96·78	69·61
0·894	68·33	19·74	0·851	84·60	48·27	0·808	97·02	70·03
0·893	68·72	20·42	0·850	84·93	48·84	0·807	97·27	70·46
0·892	69·11	21·11	0·849	85·26	49·38	0·806	97·51	70·88
0·891	69·50	21·79	0·848	85·59	50·00	0·805	97·73	71·26
0·890	69·92	22·53	0·847	85·94	50·61	0·804	97·94	71·64
0.889	70·35	23·29	0·846	86·28	51·21	0·803	98·16	72·02
0·888	70·77	24·02	0·845	86·61	51·78	0·802	98·37	72·40
0·887	71·17	24·73	0·844	86·93	52·34	0·801	98·59	72·77
0·886	71·58	25·44	0·843	87·24	52·90	0·800	98·80	73·14
0·885	71·98	26·15	0·842	87·55	53·43	0·799	98·98	73·47
0·884	72·38	26·85	0·841	87·85	53·96	0·798	99·16	73·81
0·883	72·77	27·52	0·840	88·16	54·50	0·797	99·35	74·14
0·882	73·15	28·19	0·839	88·46	55·02	0·796	99·55	74·50
0·881	73·54	28·87	0·838	88·76	55·55	0·795	99·75	74·83
0·880	73·93	29·57	0·837	89·08	56·10	0·794	99·96	75·18
0·879	74·33	30·26	0·836	89·39	56·66		Alcohol	
0·878	74·70	30·92	0·835	89·70	57·20	0·7938	100·00	75·25
0·877	75·08	31·58	0·834	89·99	57·71			

Brandy is spirit derived from the grape. It then contains, besides ordinary ethylic alcohol, a number of secondary products, including organic acids (chiefly acetic), aldehydes (compounds midway between alcohol and acid), ethers (of which acetic is the more important), furfural, and higher alcohols

(including amylic, which constitutes the chief constituent of so-called fusel oil).

The *Lancet* Analytical Commission found brandy to have the following composition (in grammes per 100 litres of alcohol present):

Acidity (in terms of acetic acid), 64 to 85 grammes.
Aldehydes, 10 to 14 grammes.
Ethers (in terms of ethyl acetate), about 100 grammes.
Furfural, 1·6 to 2·6 grammes.
The alcoholic strength is about 50 per cent. by volume.

Grain-spirit, beet-spirit, and gin contain only very small quantities of ethers; rum contains ethers in great excess; in whisky the ethers, though less, more nearly approximate to brandy. The proportions of other secondary products vary in these liquids from those of genuine brandy.

Spurious brandy is mainly derived from rye, potatoes, maize, barley, and figs; and the alcohol may be distilled over in either a pot-still or patent-still. The pot-still consists of a body, termed the "pot," with a long neck at the top. The neck is connected with a tortuous pipe, termed the "worm," which, by means of a constant stream of water, acts as a condenser. The spirit is highly rectified by the patent or fractionating still, whereby secondary products are almost entirely removed.

The raw materials employed in the production of modern **whisky** are malt and grain (chiefly maize), with small quantities of wheat and oats; and the fermentative action of the yeast, by which the maltose is split up into alcohol and carbonic acid, is pushed to the extreme limit, with the object of converting all the sugar into alcohol. Secondary products in patent-still whisky are present in very small quantity, but they are greater in genuine pot-still whiskies; and it has been suggested that the minimum "coefficient" of secondary products in the latter whiskies should be taken at 380 parts per 100,000 parts of absolute alcohol, and the ethers at 80.

Furfural gives an easily obtained and very distinct colour reaction; it is certainly the most toxic constituent of whisky. Besides the secondary products mentioned above, whisky also contains other bodies, concerning the chemical nature of which we are ignorant, and it is probably to them that it owes its characteristic taste and flavour. Whisky, therefore, differs from

brandy not only in taste and flavour, but also in that it contains relatively more higher alcohols and relatively less compound ethers; it further contains traces of empyreumatic or tarry substances derived from the malting process. Concerning the therapeutic action of the secondary products in brandy and whisky—namely, the higher alcohols, the ethers and aldehydes—we have very little exact information; but some authorities maintain that they are serviceable for medicinal purposes.

The higher alcohols and ethers in wine are generally present in much the same ratio as in brandy.

The *ethers* present in genuine brandy usually amount to about 100 parts per 100,000 of the absolute alcohol present. (This method of calculating on the absolute alcohol present enables a comparison to be made between different brandies which may vary in their alcoholic strength.)

The estimation of the ethers is performed as follows: Having obtained the distillate and, from the specific gravity, ascertained the amount of alcohol (as previously described), a drop of phenolphthalein solution is added to the distillate, and any free acid that may have come over with the alcohol is exactly neutralized. It is then transferred to a hard glass boiling-flask, 25 c.c. of $\frac{N}{10}$ alcoholic soda added, and complete saponification is effected under a reflex condenser in about two hours. Then, after cooling, titrate the excess of soda with $\frac{N}{10}$ hydrochloric acid. Each c.c. of soda which has been used in saponifying the ethers corresponds to 0·0088 gramme of ethyl acetate. It is desirable to make a blank experiment with pure spirit.

Furfural.—To test for this, 10 c.c. of distillate should be diluted to 50 per cent. of alcohol, and placed in a glass cylinder. Ten c.c. of standard furfural (0·005 gramme per litre) is placed in a similar cylinder. To each, 10 drops of pure colourless aniline oil and 1 c.c. of pure acetic acid are added. Furfural strikes a bright reddish-pink colour; and after fifteen minutes the colour in the two cylinders may be compared and an approximate estimate of the furfural in the spirit thereby arrived at.

Fusel oil is a mixture of oily bodies consisting chiefly of pentylic or amylic alcohol, and is a constant accompaniment of common alcohol. It is a product which appears to be, bulk for bulk, more injurious than ordinary ethylic alcohol, and it should not be permitted to exceed 0·2 per cent.

Acidity.—The acidity of brandy is about 0·05 to 0·11 per cent., that of whisky about 0·1 per cent., and that of rum about 0·5 per cent.

Detection and Estimation of Fusel Oil (Amylic Alcohol).

The presence of fusel oil may also be detected by (*a*) slowly distilling off the great bulk of the liquid and extracting the residue in the flask with ether; the ethereal solution is allowed to evaporate spontaneously, and then the residue is heated with sulphuric acid and sodium acetate, when the odour of pear is evolved.

(*b*) By decolorizing with animal charcoal, adding a few drops of hydrochloric acid, and afterwards some fresh and colourless aniline oil; in the presence of fusel oil the aniline compound acquires a rose tint.

Röse's quantitative estimation is based on the following facts: Chloroform possesses the property of rapidly removing amylic

FIG. 66.—APPARATUS FOR ESTIMATING FUSEL OIL BY ROSE'S PROCESS.

alcohol from its solution in diluted spirit, and the presence of amylic alcohol in chloroform increases its power to dissolve ethylic alcohol. When, therefore, chloroform is shaken with diluted ethylic alcohol containing amylic alcohol, there will be a notable increase in its volume.

The apparatus required is a stoppered tube (Fig. 66), capable of holding about 180 c.c., having the lowered part, holding about 50 c.c., drawn out and graduated.

Twenty c.c. of chloroform are introduced into the bottom part

of the tube by means of a long-necked funnel, so that it shall not collect on the upper sides of the tube. The spirit to be tested is first diluted or strengthened until it has a S G. of 934·6 at 15° C.—*i.e.*, contains 50 per cent., by volume, of real alcohol —and 100 c.c. of this prepared spirit is carefully run on to the top of the chloroform. The stopper is greased with vaseline and tightly fitted, and the whole tube immersed for an hour in water for the chloroform to settle (which process is aided by occasional tapping). After an hour the volume of the bottom layer is read off; if the spirit is pure, this volume will now be 37·1 c.c., but if it contains 1 per cent., by volume, of amylic alcohol, the bottom layer will measure 39·1 c.c.; thus giving an increase of 1 c.c. for each ½ per cent., by volume, of amylic alcohol in the sample.

When this process is applied to the ordinary raw spirits of commerce, the results are somewhat below the truth, on account of the presence of other impurities, which have, however, less tendency to pass into the chloroform.

The commonest form of *adulteration* of spirits is by the addition of water. When the distillate gives a decided red colour within fifteen minutes with 1 per cent. nitro-prusside of sodium solution and ammonia, methylated spirit is present. The tests of taste and odour alone afford an excellent index to purity.

Whisky, like brandy, is frequently coloured with caramel.

Gin is comparatively free from fusel oil. It is made from cereals, and flavoured with juniper berries, etc.

Rum is generally obtained by distilling fermented molasses.

WINE.

Wine is the fermented juice of the grape. The amount of alcohol depends on the amount of glucose present in the grape-juice. One molecule of glucose forms two of alcohol and two of carbonic acid ($C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$).

The different kinds of wine, and even the different makes of the same wine, vary considerably in their composition. The alcohol generally constitutes from 8 to 18 per cent. by weight, the solid residue from 2 to 6 per cent., and the mineral ash constitutes about 0·2 per cent. Mineral ash below 0·15 per cent. would justify suspicion.

The estimation of the acidity is of importance. In wine the

acidity is generally returned in terms of *crystallized tartaric acid per cent.* The sample should be diluted before titration, well shaken to remove any CO_2 present, and phenolphthalein used as indicator. Every c.c. of the decinormal soda required for neutralization=7·5 milligrammes of crystallized tartaric acid. The acidity—which is chiefly due to such acids as tartaric, malic, acetic, formic, and butyric—should not exceed 1·2 per cent. of tartaric acid. It very rarely exceeds 0·8 in genuine samples, and is seldom below 0·4. About $\frac{1}{4}$ of the total acidity in white wines and not less than $\frac{1}{3}$ in red wines should be due to volatile acids. The volatile acids may be estimated as follows (Windisch): The total acidity in 25 c.c. of the wine is estimated as tartaric acid; 25 c.c. of the wine are then evaporated in a china basin to a volume of 2 to 3 c.c., 25 c.c. of hot water are added, and the whole again evaporated down to 3 c.c.; another 25 c.c. of water are added, and after evaporation the residue is dissolved in hot water, and the solution titrated. The result, calculated as tartaric acid, is the non-volatile acidity. The difference between this and the total acidity gives the volatile acidity as

FIG. 67.—TORULA CEREVISIÆ (YEAST PLANT). (× ABOUT 200.)

tartaric acid. The tartaric acid is converted to acetic acid by multiplying by 0·8 (since tartaric acid is to acetic acid as 7·5 is to 6·0), for the volatile acidity is always expressed as acetic acid.

The commoner adulterants are water, sugar, various ethers, logwood, sulphate of lime, and alum; and these are, of course, especially employed in the manufacture of the cheaper wines. Calcium sulphate is used to improve the appearance of wines by clarifying and furnishing a brighter and more permanent colour, and it also improves the keeping qualities. The grapes are sprinkled with sulphate of lime either before or after they are put into the vat. This so-called "plastering" may be injurious to health; it is indicated when the SO_3 in 100 c.c. exceeds 0·092 gramme; it gives rise to the formation of potassium sulphate, which has a decided purgative action. In France this salt is

not permitted to exceed 0·2 per cent. If 50 c.c. of the wine are acidified with HCl and precipitated hot with barium chloride, the sulphate found may be calculated as potassium sulphate. Sodium chloride is sometimes added; it should not exceed 0·05 per cent. The ash of genuine wines falls between 0·15 and 0·35 per cent.

Astringent agents commonly employed are tannin, alum, and catechu.

Boric acid and salicylic acids are often added; they may be normal constituents of wine in minute quantities. Fluorine and saccharine may also be added to improve the keeping qualities of wine.

Already methods have been given by which most of the adulterants mentioned may be detected. The tannin in a measured quantity of wine may be estimated as suggested by Kramsky. From 50 to 100 c.c. of the wine are rendered alkaline with ammonia and precipitated with a solution of zinc hydroxide. The zinc reagent is prepared by dissolving 25 grammes of zinc sulphate in water, adding sufficient ammonia to redissolve the precipitate formed, then 300 c.c of ammonia, and finally water to make the volume up to 1 litre. The precipitate of zinc tannate is stirred until it coagulates and settles. Three hundred c.c. of hot water are added, and the precipitate is collected on a weighed filter, washed with dilute ammonia, dried at 100° C., and weighed. The filter and precipitate are now ignited, and the weight of zinc oxide obtained is subtracted from the total weight, the difference giving the amount of tannin. Gallic acid is not precipitated by the above reagent, and the ordinary constituents of wine have no influence on the estimation. In genuine samples of red wine the tannin does not exceed 0·25 per cent., and it is less in white wines.

The colouring agents which have been employed are logwood, blackberry, elderberry, and prune juices; sandalwood, cochineal, magenta, Brazil wood, aniline reds and violets, and indigo; they are practically harmless; some of these colours are heightened in tone by tartaric acid.

For analytical purposes "white" and "red" wines may be almost decolorized with animal charcoal, and "red" wines with basic acetate of lead and magnesium sulphate.

Many tests have been suggested for the detection of foreign colouring matter in wine, but few of them are found to be invariably satisfactory in practice; two of the best are—

(*a*) A 10 per cent. solution of good clear gelatine is allowed to set, when from the firm mass several small cubes of about $\frac{3}{4}$ inch square are cut, and two or three of these cubes are immersed in the wine for twenty-four hours. In pure wines the colouring matter does not penetrate the gelatine for more than about $\frac{1}{16}$ of an inch, but the majority of the foreign colouring matters penetrate almost, if not quite, to the centre of the cubes. Dilute ammonia will dissolve out the colouring matter of cochineal and logwood, and will strike a blue with alkanet (Dupré).

(*b*) Fifty c.c. of the sample are mixed with 1 c.c. of 40 per cent. formaldehyde and 4 c.c. of hydrochloric acid, and heated for a few minutes on the water-bath until a precipitate begins to form. A slight excess of ammonia is then added, and the heating continued until the free ammonia has disappeared. Genuine wines give a colourless filtrate, whilst those which have been coloured artificially retain the colour of the dyes (Trillat).

The colour of genuine wine does not dialyze to any marked extent, but that of logwood, cochineal, and Brazil wood does so readily.

The presence of foreign colouring agents having been ascertained, it remains to discover their nature by special and appropriate tests. Some of these are indicated in the chapter on Antiseptics and Colouring Agents in Food.

Wines are sometimes fortified with inferior brandies.

It may be necessary to examine any of these beverages for poisonous metals, which may be introduced by the use of lead and zinc utensils in their manufacture, the cleansing of bottles with shot, etc. The darkening of wine which sometimes results from the presence of iron is not objectionable.

The demand for white wine has increased during recent years, and some producers of red wines have set about to find means to convert it into white. Analyses made in France prove that one mode of effecting this is to treat red wine with a mixture of animal charcoal and potassium permanganate. This process bleaches the wine, but at the same time leaves a considerable quantity of manganese in solution. Wine which has thus been treated can be recognized by adding to it in an open vessel an excess of caustic soda, and shaking. After a few minutes a thin brown layer forms on the surface of the liquid, due to oxidation by the air of the oxide of manganese set at liberty by the alkali.

British wines, sold as non-alcoholic, frequently contain salicylic acid, and sometimes boric acid. They may contain alcohol up to 10 per cent. by volume.

Beer.

This beverage was formerly made from malt and hops only; now it can be legally made from starch and sugar and various vegetable bitters.

From the malt the beer derives maltose, dextrine, albuminoids, and salts, and from the hops a bitter principle, resin and tannin; alcohol, carbonic acid, a little glycerine, and succinic acid are produced by the fermentation, and a small amount of lactic and acetic acid results from schizomycetic fermentation.

Pure beer is the fermented liquor obtained from the germinating grain of barley. The grains are made to partially germinate by being first moistened and then kept warm until they begin to sprout. A small quantity of the ferment "diastase" is thus produced, and the diastase acts upon the starch and largely converts it into the sugar "maltose," which is easily fermentable. Further fermentation is then prevented by heating the barley in kilns. The malt is next subjected to "mashing" by mixing with water at 82° C. and well crushing and stirring for about two hours. After clarifying, the infusion is boiled with hops, and the cooled liquor or "wort" is transferred to vats to ferment, yeast being added. When the alcoholic fermentation has proceeded far enough, the yeast is removed and the beer is run into casks.

In recent years glucoses and invert sugars obtained from rice and other starches by the action of dilute sulphuric acid have been largely substituted for the malt. The commercial sulphuric acid employed is liable to contain arsenic (derived from the iron pyrites used in its manufacture); and this circumstance was responsible for a considerable outbreak of arsenical poisoning among beer consumers, chiefly in the north-western part of England, in the winter of 1900-1901. Amounts of arsenic varying from $\frac{1}{500}$ to 1 grain per gallon of beer were discovered, and some invert sugars were found to contain arsenic equivalent to 2·04 grains of arsenious oxide per pound.

With regard to the "finings," which are added to clarify beer or wine, their nature is varying, but they generally contain isinglass.

Sulphites or bisulphites, and carbonates or bicarbonates of soda and potash, sulphurous, boric, and salicylic acids, and saccharin, are employed, principally to check fermentation and to clarify. The use of sulphites has increased of late years, while that of salicylic acid has decreased. Fluorine may also be employed.

An undue amount of common salt may be present. Before it can be decided that salt has been added, allowance must be made for the salt in the brewing water, and for the chlorides natural to the malt and hops. Fifty grains of salt per gallon would be a most generous limit to allow on these accounts, but where possible the chlorine in the actual water used in the brewing should be estimated.

Sulphurous acid may find its way into beer (and wine) from the practice of sulphuring the insides of the casks, and washing them with a solution of calcium bisulphite or sulphite of potassium; but it is also added to regulate fermentation and to produce a flavour of age. It may be detected by adding to some of the beer or wine HCl and zinc powder, and then laying a lead paper over the mouth of the bottle; the paper darkens if sulphurous acid is present ($SO_2 + 3H_2 = SH_2 + 2H_2O$).

Alkaline salts are added to correct undue acidity. Sodium bicarbonate has also been employed to increase the effervescing property of the beverage.

Other bitters (quassia, catechu, and tannin) are sometimes used as substitutes for hops; but this substitution has no public-health import. Noxious bitters have in former times been detected, such as picrotoxine, nux vomica (strychnine), and picric acid.

The acidity of beer is an important consideration, since excess either denotes commencing changes of a deteriorating character, or implies the addition of sulphuric acid (employed to clarify, to lighten the colour, and to give the beer the hard taste which naturally only comes by age). The normal acidity of beer depends upon the presence of carbonic, acetic, lactic, malic, tannic, and gallic acids, and it can be estimated in terms of *glacial acetic acid* by neutralization with decinormal soda solution (1 c.c. of which=6 milligrammes of glacial acetic acid). The acidity of 100 c.c. of beer should not exceed 30 c.c. of decinormal alkali.

The ash should not be less than 0·12 nor more than 0·40

gramme per 100 c.c.; if the latter figure is exceeded, probably some mineral adulterant has been added.

A neutral solution of lead acetate will largely decolorize beer.

Herb and botanic beer and ginger beer, which are sold as "non-alcoholic" beverages, often contain proof-spirit in excess of the limit of 2 per cent. A few of the many samples of ginger beer analyzed at the Government laboratories during the years 1905, 1906, and 1907 contained 6 per cent. of proof-spirit—one sample containing 9·5 per cent.; a sample of herb beer contained 10·5 per cent. of proof-spirit, and one of dandelion stout 12·3 per cent.

CHAPTER X

VINEGAR—LIME AND LEMON JUICE—MUSTARD—PEPPER —SUGAR—HONEY

VINEGAR (ACETIC ACID).

THIS is the acid liquid obtained from the acetous fermentation of various decoctions or fruit juices. Wine, cider, and malt vinegars are due to the oxidation of alcohol by the action of *Mycoderma aceti*. The bulk of the vinegar used in this country is derived from malt and barley, in France from wine, and in the United States from cider. Spirit vinegar is made in Germany, and small quantities reach this country.

Vinegar is essentially dilute acetic acid, generally 4 to 5 per cent., along with a little acetic ether. Vinegars made chiefly from unmalted barley, maize, rice, and other grains, and from sugar or molasses, are sometimes sold as malt vinegar. Pyroligneous acid (wood acid) has been employed to make up vinegar; it is derived from the destructive distillation of wood, and often a little caramel is added to make it resemble malt vinegar.

In this country there are no legal standards relating to vinegar, but "a pure malt vinegar" should contain nothing but what is furnished to it by the barley, the yeast, and the water which are used in its manufacture.

The Local Government Board is of opinion that the following definitions may properly be adopted:

Vinegar is a liquid derived wholly from alcoholic and acetous fermentations. It shall contain not less than 4 grammes of acetic acid ($C_2H_4O_2$) in 100 c.c. of vinegar; it shall not contain arsenic in amounts exceeding 0·0143 milligramme per 100 c.c. of vinegar, nor any sulphuric or other mineral acid, lead, or copper, nor shall it contain any foreign substance or colouring matter except caramel.

The practice of adding mineral acid to brewed vinegar is said to have ceased; but free sulphuric acid is sometimes added to cheap vinegars, made by the addition of acetic acid to water.

Free sulphuric acid may be detected by Ashby's test: Dry a drop of the aqueous extract of logwood (0·5 gramme to 100 c.c. of boiling water, and allowed to stand for a few hours) on a porcelain plate, add a drop of the vinegar, and again dry. Pure vinegar gives a yellow residue, but if free mineral acid is present the residue is red.

Mineral acids may also be detected by adding 4 to 5 drops of a 0·1 per 1,000 solution of methyl violet; pure acetic acid vinegar shows no change of colour, but traces of free mineral acid cause the violet colour to change to a bluish-green or green.

To estimate the quantity of sulphuric acid present, mix 50 c.c. of the vinegar with 25 c.c. $\frac{N}{10}$NaHO in a platinum dish, evaporate to dryness, and ignite at a low heat. Add 25 c.c. $\frac{N}{10}$ HCl to neutralize, heat to expel CO_2, and filter. Wash out with hot water, adding washings to filtrate. Estimate free acid present with $\frac{N}{10}$ NaOH, using phenolphthalein as the indicator. The number of c.c. used × 0·0049=amount of free H_2SO_4 in 50 c.c. of the sample (Otto Hehner). Some allowance must be made for the sulphates in the water used in the manufacture of the vinegar.

If the *ash* of vinegar is alkaline (from the conversion of the organic salts into carbonates by the ignition), this shows that no free sulphuric acid was present.

The amount of sulphuric acid can be estimated, as in water, by precipitating as barium sulphate. The precipitate is then collected, washed, dried, ignited, and weighed, and the weight multiplied by 0·343 gives the weight of SO_3.

The *acidity* of vinegar should not fall below 4 to 5 per cent. of glacial acetic acid ($C_2H_4O_2$), and the specific gravity does not fall below 1015 in good vinegar. In artificial vinegars made by diluting acetic acid the specific gravity is generally about 1007, and the mineral ash only about 0·035 (as against 0·35 in brewed vinegars).

To estimate the total acidity of vinegar, take 10 c.c., dilute it with 90 c.c. of distilled water, and into 10 c.c. of the mixture (which contains 1 c.c. of vinegar) run decinormal soda solution until the neutral stage is reached, using phenolphthalein as indicator. The number of c.c. of soda solution required ×·0·00 × 6100=the percentage amount of acetic acid present.

Potassium ferrocyanide has been used to clarify, and a little hydrocyanic acid results. This enters into unstable combination with the organic constituents, and although no ill-effects to consumers have been observed the practice is objectionable.

The preservative now chiefly employed is calcium bisulphite, which may be added to the finished vinegar, or may gain access to the vinegar from its use for cleansing vessels. Salicylic acid and boric acid are also sometimes employed.

In cases where vinegar has been added to "tinned" articles (pickles, fish, etc.), the liquid should be tested for metals, since the vinegar adds considerably to the solvent action of the juices. Copper may gain access from apparatus employed in the making of vinegar; but metallic pumps and pipes are now generally superseded by ebonite. Traces of arsenic may also be found when arsenical malt has been employed.

The detection of heavy metals is difficult in the case of highly coloured vinegars. The following method is recommended for lead and copper: 1 c.c. of concentrated hydrochloric acid is added to 10 c.c. of the boiling vinegar, and the liquid treated little by little with potassium chlorate until of a pale yellow colour, after which it is boiled for a minute, treated with sodium acetate, and subjected to a current of H_2S.

Vinegar eels (*Anguillula oxyphila*) are not considered injurious.

LIME-JUICE AND LEMON-JUICE.

Lime-juice is the juice of *Citrus limetta* fortified with about 1 ounce of brandy to every 10 ounces of juice. The specific gravity (at 15° C.) is generally about 1037, the alcohol forms about 4 per cent., and the calculated free citric acid is about 30 grains to the ounce (1 c.c. of decinormal soda solution=6·9 milligrammes of citric acid. The percentage amount of acid ×4·375 gives grains per ounce).

The ash of lime-juice dissolved in neutral distilled water is alkaline in reaction.

Adulteration.—The juice may be entirely made up of citric acid flavoured with essence of lemon; or of citrate of potash, tartrates, the juices of plants, etc. Sulphuric acid is sometimes added, and also water. The usual determinations made are: the acidity, the alkalinity of the ash, the fragrancy of the extract, the physical characters (agreeable odour and taste, amber colour,

and clearness), and the freedom from sulphuric and tartaric acids.

To test for tartaric acid, take 2 grammes of the lime-juice, and dissolve in 45 c.c. of proof-spirit (methyl spirit diluted to a density of 920). Then add 5 c.c. of a cold saturated solution of potassium acetate in proof-spirit, and stir for ten minutes, when a crystalline precipitate of the acid tartrate of potassium forms. If the precipitate is small in amount, only white streaks may be observed on the side of the vessel in the track of the glass stirring-rod.

In estimating acidity, it is necessary to first considerably dilute the juice.

Lemon-juice, according to the British Pharmacopœia, should have a specific gravity of 1030 to 1040, should contain 30 to 40 grains of free citric acid per ounce, and should not yield more than 3 per cent. of ash. The B.P. figure for citric acid is too high. The Board of Trade standard is a specific gravity (without spirit) of not less than 1030, and an acidity equal to 30 grains per ounce of citric acid.

These juices should be tested for salicylic acid, sulphites, and boric acid.

MUSTARD.

The mustard in general use is practically a mixture of brown and white mustard-seed ground to flour.

None of the adulterants employed are of a harmful nature They are: Turmeric and aniline yellow, wheat starch, and whe[n]

FIG. 68.—THE CUTICLE OF THE WHITE MUSTARD-SEED. (× 200.)

much foreign material is added, aniline dye and a little cayenne pepper may be employed. The brownish-red reaction of turmeric with ammonia, and the blueing of starch in the presence of iodine after boiling some of the sample with distilled water and allowing to cool, are chemical tests of service, for pure mustard contains no starch or turmeric.

Mustard oil is sometimes abstracted.

White mustard under the microscope presents certain well-marked characteristics. The outer coat, or cuticle, consists of a layer of large hexagonal (so-called "infundibuliform") cells, which present a central ostium occupied by other bodies, called mucilage cells. When water is added these latter swell up, and escape from the mouth of the large hexagonal cells into the water, to which they appear to furnish mucilage.

Inside this layer are three less characteristic ones, the innermost consisting of a thin layer of large granular cells; and the interior of the seed comprises a fairly regular areolar network, containing granular matter and minute oil globules.

Mustard polarizes well.

PEPPER.

White pepper is obtained from the pepper berry after the dark outer layer of pericarp has been removed. This accounts for the difference in the composition of black and white peppers.

Pepper is considerably adulterated, but mostly with agents which are harmless. Various starches (rice chiefly) and the ground stones from olives ("poïvrette") have been employed.

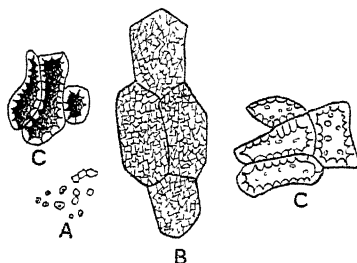



FIG. 69.—PEPPER. (× 250.)
A, Isolated starch grains; *B*, cells of the perisperm with starch; *C*, schlerenchymatous cells.

Any added mineral matter could, as in flour, be mostly separated by shaking up thoroughly with chloroform. A little sand or earthy matter is general, owing to the fact that the berries are allowed to dry on earthen surfaces, when many of their minute furrows become filled with the earth; and by the wearing of the stones between which the berries are ground the powder may also acquire a trace of sand. A very small allowance has to be made for the presence of this *unavoidable* mineral matter.

A large proportion of the pepper sold has been reduced in quality by being bleached.

A rather rough but serviceable test, recommended by Neuss, consists in covering the pepper with concentrated hydrochloric acid, when, if the sample is pure pepper, it becomes of an intense and uniform yellow, while most of the foreign ingredients remain uncoloured. The microscope furnishes a useful means of detecting adulteration.

The ash of black pepper averages from 3 to 5 per cent.; that from white pepper 1 to 3 per cent.

According to the resolution of Bavarian chemists (1890), the limits of ash should be:

For black pepper, 6·5 per cent. (2 per cent. insoluble in HCl); for white pepper, 3·5 per cent. (1 per cent. insoluble in HCl).

Unlike mustard, pepper contains starch; but this is not the case with "poivrette."

Microscopical Characters of Pepper.—A transverse section of the *black-pepper* berry shows the following notable points: Starting from the cortex, most externally is a layer, two or three cells deep, arranged vertically, and very much resembling bean-starch granules in appearance—*i.e.*, ovoid in shape, with a central linear hilum crossed by transverse markings; next follows an ill-defined layer of elongated cells arranged transversely to the foregoing, and then a sort of irregular reticular tissue containing oil globules; more internally still, a well-defined single layer of large vertical more or less flask-shaped cells is seen. The rest of the interior of the berry consists of flattened angular cells, dovetailed into each other.

The following is a chemical test for "poivrette": Digest for forty-eight hours 1 gramme of phloroglucol in 50 c.c. of hydrochloric acid (S.G. 1·1), and then decant the clear solution. Just cover some of the pepper with this reagent, and heat cautiously until fumes of hydrochloric acid begin to come away. "Poivrette" furnishes a deep cherry-red colour, but pepper a yellow or faint brown.

"Long pepper" is very inferior in strength to other kinds. It consists of the fruit of *Chavica Roxburghii*, and it usually contains a considerable quantity of extraneous mineral matter (clay and soil). It may be used to adulterate pepper.

By polarized light an entirely dark field may be obtained with

pure pepper on rotating the prisms, but this is not possible when "poivrette," "long pepper," or rice is present.

Cayenne pepper has been adulterated with oxide of iron, brick-dust, rice, starch, etc. The mineral ash should not exceed 6·5 per cent., and not more than 0·5 per cent. of the ash should be insoluble in HCl.

Other Spices.

Ginger is sometimes adulterated with "exhausted" ginger which has been used in the manufacture of ginger beer or ginger wine. The soluble ash should be at least 1·5 per cent. *Caraways* and *cloves* which have had the essential oil extracted from them may be mixed with the pure commodity; and ground mixed spices may be adulterated with damaged macaroni and vermicelli, or walnut shells, olive stones, etc., ground up into a powder.

Sugar.

The sugar of commerce is almost entirely beet and cane sugar ($C_{12}H_{22}O_{11}$), for very little glucose comes into ordinary domestic employ; but sometimes beet-sugar is artificially coloured with minute quantities of aniline colours, and thus made to resemble cane-sugar.

The colouring matter foreign to sugar may generally be detected by washing about 100 grammes of the sugar in a flask with alcohol (90 per cent.). If the dye is not removed, the washing must be repeated until it is. The solution is then filtered, evaporated to dryness, and again taken up in a little alcohol. A skein of wool slightly mordanted with aluminium acetate is placed in the solution, which is warmed over the water-bath. The skein is removed after a while, washed with water, and dried, when it retains a permanent yellow dye. The colour natural to sugar will not furnish a solution which is capable of permanently dyeing wool.

Demerara sugar owes its bright tint to the natural colouring matter of the cane being fixed on the sugar by means of a mordant of chloride of tin. To imitate this, beet-sugar crystals are sometimes stained with an aniline dye. This can often be detected by merely moistening the sugar with a little strong hydrochloric acid, which turns the yellow dye to a bright pink, but does not affect the natural colour.

Ultramarine blue is sometimes added to granulated and loaf sugars to improve their colour. The sample should be dissolved in water, and the colouring matter allowed to settle, when it can be collected and washed with water. HCl discharges this colour and liberates sulphuretted hydrogen.

In estimating the ash of sugar it is best to carefully ignite 5 grammes of the powdered sugar. after mixing (by means of a glass rod) with 5 to 7 grammes of coarsely powdered quartz sand (previously ignited and weighed). The platinum dish may, however, be perceptibly injured in the process. The ash of genuine sugar does not exceed 2 per cent. Carbonate of calcium may be found, as it is said to be used in the

FIG. 70.—THE SUGAR MITE (ACARUS SACCHARI). (MAGNIFIED.)

whitening of sugar (A. Hill). Any insoluble mineral adulteration would be detected by dissolving the sugar in water and then filtering.

The amount of sugar present in any substance is best estimated by Fehling's method, the rationale of which is as follows: If solutions of caustic potash and sulphate of copper be boiled together the mixture becomes black, owing to the formation of the *black* oxide of copper. The presence of certain organic substances, however, and amongst them grape-sugar, prevents the copper becoming so highly oxidized, and the *reddish-brown* sub-oxide of copper is in consequence formed. Cane, beet, and maple sugar ($C_{12}H_{22}O_{11}$) have no such action, but by heating the clarified syrup (containing not more than 25 grammes of the solid per 100 c.c.) in a water-bath along with about one-tenth of its bulk of strong HCl for fifteen minutes—or by boiling with a 2 per cent. solution of citric acid—it is readily inverted into glucose ($C_6H_{12}O_6$). Before, however, the inverted sugar is estimated by Fehling's method, the acid solution must be neutralized.

22

By inversion, then, is understood the conversion of non-reducing carbohydrates into sugars of the formula $C_6H_{12}O_6$, which directly reduce Fehling's solution to a reddish-brown sub-oxide of copper.

Levulose ($C_6H_{12}O_6$) or fruit-sugar, maltose ($C_{12}H_{22}O_{11}$) or malt-sugar, and lactose ($C_{12}H_{22}O_{11}$) or milk-sugar, reduce Fehling's solution at once.

Fehling's solution is made by dissolving 34·64 grammes of pure cupric sulphate in water and diluting to 500 c.c. The solution so obtained is mixed with another solution prepared by dissolving 173 grammes of sodio-potassic tartrate in water, adding 100 c.c. of sodic hydrate solution (S.G. 1·34), and diluting the mixture to 500 c.c. When these two solutions (each of 500 c.c.) are united, we obtain 1 litre of ordinary Fehling's solution.

The solution should be preserved in small, well-stoppered bottles kept full and in the dark.

The estimation of sugar by Fehling's method is performed as follows:

Take 50 c.c. of Fehling's solution, bring to the boil in a porcelain dish, and then run in that amount of very dilute (about 1 per cent.) sugar solution which, when boiled for two minutes, decolorizes the Fehling's solution. Then filter the hot solution. If the filtrate is bluish-green, far too little sugar has been added; if it is greenish, barely sufficient sugar has been added; if yellow, either the right quantity or too much sugar has been added. In the case of the filtrate being yellow a little copper may still exist in solution. To detect this, acidify with acetic acid, and add a drop or two of potassium ferrocyanide; a bronze coloration will indicate that barely sufficient sugar has been added.

Thus by a series of experiments the precise amount of sugar solution required to reduce 50 c.c. of Fehling's solution is arrived at. Suppose 25 c.c. of the sugar solution are required. Then 25 c.c.=50 c.c. of Fehling's solution=0·2375 gramme of grape-sugar; for, according to Soxhlet, if we work with dilute solutions containing as near as possible 1 per cent. of sugar, 50 c.c. of Fehling's solution are reduced by—

0·2375	gramme of	grape-sugar.
0·2470	,,	invert-sugar.
0·2572	,,	levulose.
0·3380	,,	lactose.
0·3890	,,	maltose.

Ling and Rendle's indicator is a very useful reagent for determining the complete reduction of Fehling's solution. It is made as follows: 1 gramme of ammonium thiocyanate and a similar quantity of ferrous ammonium sulphate are dissolved in 10 c.c. of warm distilled water; then 5 c.c. of concentrated hydrochloric acid are added. This produces a dark brown solution, which must be decolorized before using. To effect this a trace of zinc dust is added to about 2 c.c. of the stock solution.

About a dozen drops of the indicator should be placed upon a white porcelain slab, and drops from the dish containing the boiling Fehling's solution should be transferred by means of a glass rod to the drops on the slab. If a bright blood-red colour is struck, reduction is not yet complete; otherwise, no colour results. In another simple method (W. F. Sutherst) a drop of the mixture is placed on the top side of a filter-paper folded in half; the filtrate passes through, and the spot is treated with a drop of a dilute acetic acid solution of 1 per cent. potassium ferrocyanide. On holding up to the light, the faintest trace of copper ferrocyanide is plainly seen, and the end of the reaction readily indicated.

It is important to realize that milk-sugar takes a much longer time to reduce Fehling's solution than glucose does.

The use of glucose in jams, jellies, confectionery, and marmalades cheapens these products, and it serves to prevent crystallization of cane-sugar; hence it is largely employed, although it is not essential to the production of these preserves. It is also employed in sundry beverages, particularly brewed ginger beer and certain kinds of wines.

Glucose is the chief adulterant of golden syrup. Calcium sulphate is a common impurity in commercial glucose, and its presence may sometimes be detected by obtaining a filtered solution of the sample and noting a distinct turbidity on adding ammonium oxalate.

Honey.

The microscope, by revealing the absence of pollen grains, would give a certain indication of any artificial comb prepared from paraffin wax, etc. Beeswax carbonizes by treatment with boiling strong sulphuric acid; not so paraffin wax. Glucose, cane-sugar, low-grade malt extracts, and different starches have been employed to adulterate honey. Honey containing starch

syrup is coloured red to violet by iodine solution; not so pure honey.

Honey has given rise to poisoning in New Zealand and elsewhere, the poison being derived from poisonous plants the precise nature of which has not been determined.

Genuine honey should not contain more than 8 per cent. of cane-sugar, 25 per cent. of moisture, and 0·5 per cent. of ash.

JAMS.

Certain jams may be adulterated with apple-pulp or other cheaper fruits. For sweetening, starch-glucose may be employed instead of cane or beet sugar, and salicylic acid or benzoic acid may be added as a preservative. Apple-pulp may be detected by boiling some of the jam with distilled water, allowing to cool, and adding iodine, when a blueing will denote the addition of apple-pulp or some other adulterant containing starch. In testing for preservatives, it should be known that *traces* of salicylic and benzoic acids exist normally in many fruits.

Wooden pips have been discovered in fictitious raspberry jam.

CHAPTER XI

COFFEE—COCOA—CHOCOLATE

THE unground coffee-bean, as it reaches the market, consists of the true substance of the bean more or less enclosed in a thin skin, which is always most evident in the furrow. These coffee-berries before use are roasted at a high temperature to develop aroma, flavour, and colour.

Bell gives an analysis of Mocha coffee, which serves to show the respective amounts of the constituents of the raw bean, and the changes in these amounts which are brought about by roasting.

	Raw Coffee.	Roasted Coffee.
Caffein	1·08	0·82
Saccharine matter	9·55	0·43
Caffeic acids	8·46	4·74
Extracted by alcohol, and containing nitrogenous and colouring matter ..	6·90	14·14
Fat and oil	12·60	13·59
Legumin or albumin	9·87	11·23
Dextrin	0·87	1·24
Cellulose and insoluble colouring matter ..	37·95	48·62
Ash	3·74	4·56
Moisture	8·98	0·63
	100·00	100·00

Very generally the caffein, fat, and moisture are found to be somewhat higher than the above figures, and the cellulose lower.

The fat should amount to at least 10 per cent., and the ash to 3 per cent.

A hot-water infusion of the ground coffee contains the oil, sugar, caffein, most of the mineral matters, dextrin, and some of the albuminous matters.

Adulteration.—Chief among the adulterants is **chicory,** which is prepared from the root of the wild endive.

The admixture of chicory with coffee is very general, for many

people will not drink coffee without chicory; but it is an illegal and fraudulent adulteration when the mixture is sold as coffee. Eighty per cent. of chicory is present in some "coffee mixtures." The substance is employed to blacken and thicken the infusion, and to give a slightly bitter flavour; but, while it has a very similar dietetic value, owing to the absence of the alkaloid, it has none of the refreshing and stimulating powers of coffee.

There are several well-marked characteristics of chicory which enable its presence to be detected.

1. It has a peculiar and distinct odour, different to that of coffee.

2. The characteristic dotted ducts of the chicory (Fig. 72) form a safe and rapid means of detection by means of the microscope, and no judgment upon the presence of chicory should ever be pronounced without this evidence.

3. Roasted chicory sinks in water *at once*, while roasted coffee floats for some time, owing to the oil in the coffee preventing the particles from being readily wetted; and in chicory the sediment in the cup is soft and pulpy, while that of coffee is hard and gritty. Moreover, the particles of chicory as they sink become almost immediately enveloped in a light brown cloud, which, forming brown streaks in the water, quickly imparts a dark colour to it. Coffee will take a much longer time to achieve a similar result—*i.e.*, from fifteen to twenty minutes; and the other sweet roots with which it has been rarely adulterated—*i.e.*, mangel-wurzel, carrots, parsnips, etc.—will require the lapse of several minutes. Sometimes a little oil is shaken up with the ground chicory, which prevents it sinking. In these cases the oil can be extracted by ether, and the material then tested as to the readiness and extent to which it colours water, and as to the character of the sediment formed.

4. A useful test is as follows: If the coffee be dried, powdered, and passed through a sieve of thirty meshes to the inch, 1 gramme infused for one hour in 20 c.c. of proof-spirit will give an infusion containing 19·4 per cent. of total solids if the sample be entirely coffee. If it is entirely chicory, the total solids will total 66·4 per cent.

5. The substitutes for coffee are practically devoid of caffein. This may be determined as in Tea.

6. Take 5 grammes of coffee, and pour upon it about 25 c.c. of boiling water and filter, then to the filtrate in a Nessler glass add

acetate of lead solution. This will throw down the colouring matter of the coffee, but leave that of the chicory; and the latter can be estimated by comparing the colour with a standard mixture containing known quantities of chicory (Albert Smith).

Roasted beans, acorns, and other starches have rarely been employed, and a microscopic examination, together with the addition of iodine to the infusion (decolorized by boiling with

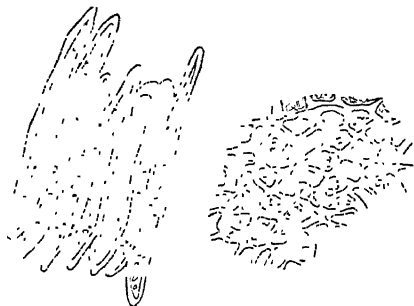



FIG. 71.—COFFEE: CELLS OF TESTA AND CELLULAR STRUCTURE. (× ABOUT 200.)

animal charcoal), would readily detect them. An infusion of *pure* chicory is not blued by iodine, nor is a strained infusion of coffee. This test may also be applied as follows: Boil the coffee for a few minutes with about 10 parts of water, allow the infusion to get quite cold, add dilute sulphuric acid, and then drop in

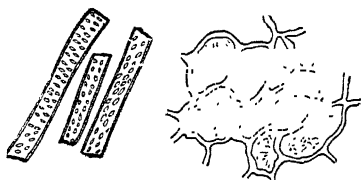



FIG. 72.—CHICORY: DOTTED DUCTS AND CELLULAR STRUCTURE. (× ABOUT 200.)

some strong solution of the permanganate of potassium with agitation, until the colouring matter is nearly destroyed, when strain and add iodine (Allen).

Burnt sugar, or caramel, has been added to improve the colour, aroma, and taste of the infusion. It can be detected by the fact that it will very rapidly and deeply colour water. A hand-lens will disclose the shining particles of caramel standing out from the comparatively dull particles of the ground berry,

and if the former are picked out with forceps they will be found quite soluble in water.

Artificial coffee-beans were within recent years placed upon the market; they were made from a paste of various starches coloured and flavoured with a little coffee and chicory. Warm water caused them to break up. Sometimes berries which have been exhausted by making coffee extract are sold.

Sugar syrup has been employed to glaze exhausted beans, and also to cause the unused berries to retain their moisture.

Coffee extracts are deficient in caffein and extractives, and may be adulterated with chicory, caramel, etc.; salicylic acid is sometimes added.

Microscopical Examination.—The microscopical characters of the raw coffee berry are distinctive. The testa or skin—portions

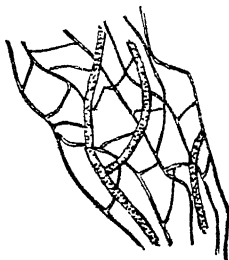



FIG. 73.—LACTEAL VESSELS OF CHICORY.

of which are always ground up with the rest of the berry—consists of long spindle cells with tapering rounded extremities, which are dovetailed into each other. Their characters, by the ¼-inch power, are shown in Fig. 71. The internal cellular substance of the berry is made up of a thick areolar tissue the meshes of which are very irregular in size and shape, and contain, in addition to starch granules, yellow angular masses and an occasional oil globule; the walls of this mesh-work are somewhat beaded in appearance.

In *chicory*, the cellular tissue, the large dotted ducts, and the lacteal vessels are characteristic. It is a good plan to soak some of the mixture of coffee and chicory in sodium hypochlorite solution and then pick out the pale membranous particles and examine these; or the mixture may be thrown on to water in a sediment flask and the particles which rapidly sink may be specially selected for examination.

The cellular tissue consists of an oval or rounded mesh-work, and is coarser than that of coffee. The dotted ducts appear as jointed tubes marked with bars and possessing extremities which do not taper. Similar structures are found in roasted beetroot. The lacteal tubes are long, pale, and narrow branching tubes, which are filled with a substance called "latex."

Cocoa.

After roasting, the husk or shell of the cocoa-seeds is cracked by machinery and then separated from the "nibs."

Cocoa-nibs, on analysis, are found to consist mainly of the following ingredients:

Fat, generally amounting to about 50 per cent.

Albuminous matter, commonly varying from 12 to 16 per cent. An astringent substance resembling tannin. Cellulose, starch, gum, cocoa-red (a substance of a resinous character, which furnishes the colour of cocoa), the alkaloid ("theobro-

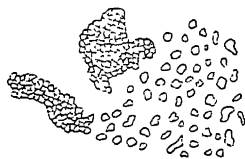



FIG. 74—COCOA STARCH CELLS. (× ABOUT 200.)

mine," which closely resembles the alkaloids of tea and coffee, amounts to over 1 per cent., and varying from 1·0 to 1·7 per cent.), mineral ash (4 to 5 per cent.), and water (5 to 6 per cent.).

About half of the ash is soluble in cold water.

"Prepared cocoa" is mixed with sugar and starch (generally arrowroot or sago), and these latter, by serving to disguise the large amount of fat, which disagrees with some persons, render it more generally preferred. Some of the fat can be readily removed from the nib by heat and pressure, and this plan is to be advocated, as the added starch does not, while it reduces the percentage of fat, provide a substance of equal dietetic value to the other constituents of the cocoa which it displaces. The microscope will at once detect the addition of such foreign starches (sago, potato, arrowroot) by disclosing their characteristic granules. Cocoa is sometimes treated with alkali ("soluble

cocoa ") with the object of emulsifying the fat so that there is less tendency for it to separate out when mixed with hot water.

An admixture with starch and sugar is permissible when the sample is not sold as pure cocoa.

The ground shells of the cocoa-seeds are sometimes added as an adulteration. The microscopic examination may detect this: and certain chemical and mechanical tests are useful.

The amounts of fat, total ash, and of the soluble ash, furnish useful tests of its purity. In the case of the ash this varies considerably in even pure samples, but it is usually under 4 per cent. The soluble ash in the cold water extract should not fall below 2 per cent. The fat in " prepared cocoa " generally amounts to from 25 to 35 per cent., but as little as 15 per cent. has been found during recent years—a circumstance which is probably due to the ready sale of the fat extracted.

Chocolate is cocoa from which much of the fat has been removed; the paste remaining is then mixed up with a considerable quantity of sugar and flavouring substances. It is liable to be attacked by the larvæ of the chocolate moth, *Ephestia elutella*. Inferior chocolate may be very deficient in cocoa.

Chocolate may be adulterated with cocoa-shell, foreign starches, or foreign fats.

Microscopically the small starch granules of *cocoa* may occasionally be seen massed together in the cellular areolar tissue, which, under a high power, is seen to be hexagonal. The appearance of the granules is shown in Fig. 74.

The most external layer of the husk of the cocoa-bean consists of long, flat, quadrangular cells; but the bulk is made up of large, distinct, and rounded mucilage cells.

CHAPTER XII

TEA—INFANTS' FOODS

TEA.

THE leaves of the tea-plant (*Thea sinensis*), as they find their way into our markets, are generally mixed with some of the flower-buds, together with numerous small stems of the plant. The difference between black and green teas is entirely due to the method of preparation, for they are both derived from the same plant.

An average sample of black tea shows about the following percentages of the two most important and characteristic constituents:

Tannin, 6 to 12 per cent.

Thein, 2 to 3 per cent.

Experiments in the *Lancet* laboratory indicate that the caffein is largely in combination with the tannin, as a caffein-tannate.

Scott Tebb has shown by experiments reproducing the conditions under which tea is ordinarily prepared for drinking that China tea generally furnishes a little less alkaloid and often nearly 20 per cent. less tannin; but that the analytical results obtained from China and Indian teas may approximate closely.

Thein is the alkaloid from which tea obtains its most valued properties; the substance has been estimated as high as 6 per cent. in some samples. The caffein of coffee and the thein of tea are apparently the same substance.

The other constituents of the tea leaf are: Cellulose, vegetable albumin, extractives (by alcohol), chlorophyll and resin, pectin and pectic acid, dextrin or gum (Bell).

An infusion of tea leaves may be made by boiling 2 grammes of powdered tea in 100 c.c. of water for one hour in a flask attached to a reflux condenser, filtering while hot, and repeating until no more colouring matter is extracted.

Such an infusion of the leaves will be found to contain the dextrin or gum, tannin, thein, most of the salts, and some of the albuminous substances, pectin, etc.

A good judge of tea will form a ready and approximately accurate estimation of its purity and genuineness by the odour and taste of a fresh infusion.

It does not appear to be an easy matter for adulteration to be practised in this connection, and it is now practically non-existent; yet formerly there were probably few articles of commerce less systematically exposed to fraudulent practices.

The *admixture with foreign leaves*, and the addition of leaves which had been already infused, were forms of sophistication

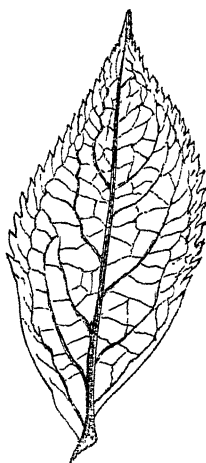



FIG. 75.—THE ELDER LEAF. (AFTER BELL.)

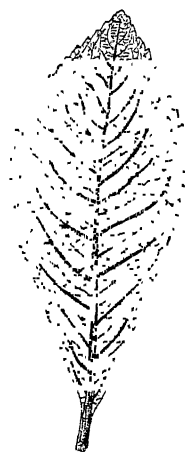



FIG. 76.—THE WILLOW LEAF (AFTER BELL.)

much practised in the past. A low magnifying power suffices to disclose the structural characters of different leaves, and fortunately the tea leaf possesses characteristics which serve to distinguish it from others; the differences are slight, however, and may be readily overlooked, especially when the leaves of the elder, willow, and sloe—which are those that have been most commonly employed—are selected to serve the purposes of adulteration.

The common method of examination is to soak the leaves in warm water, and to spread them out between glass slides, when, by holding them up to the light, all the chief characters, including the venation, can commonly be discerned if a hand-lens is em-

ployed; it is often necessary, however, to proceed to a low power of the microscope before a definite conclusion can be arrived at.

It assists in the detection of the characteristic structure of the leaves if they are previously soaked in a warm solution of sodium hypochlorite.

The characters of the *tea* leaf, when thus examined, are these: The shape is elliptical; it averages from about 1 to 2 inches in length and from ½ to 1 inch in breadth, the margin of the leaf shows distinct serrations, each of which is surmounted by a small spine, and these serrations do not quite extend to the point of attachment of the stalk; the apex is slightly emarginate; the primary veins come off dichotomously from the midrib, and then,

FIG. 77.—THE SLOE LEAF. (AFTER BELL) FIG. 78.—THE TEA LEAF. (AFTER BELL.)

branching off, form a markedly looped network extending to near the margin of the leaf, where, by bending back, they leave a narrow clear space.

Under the microscope the leaf shows an epidermic layer of flattened cells possessing well-marked sinuous outlines; coming off from a few of these cells are long slender unicellular hairs. On the under surface of the leaf there are many oval stomata visible (Fig. 79).

The presence of long-branched cells, called *idioblasts*, will also serve to identify tea from the leaves which have been most commonly used to adulterate it. A section through the thickness of the leaf should be cut near the midrib; the section should

be soaked in strong potassic hydrate solution and mounted and examined with the ¼-inch power, when the appearance shown in Fig. 80 will be observed in the case of the tea leaf.

The most distinctive characteristics of the tea leaf are: the spine-mounted serrations, which terminate a little before the point of attachment of the stalk; the looped venation; the

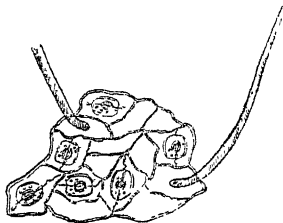



FIG. 79.—THE EPIDERMIS OF THE UNDER SURFACE OF THE TEA LEAF, SHOWING HAIRS AND STOMATA. (× 250.)

notched apex; the long slender unicellular hairs; and the large number of stomata upon its under surface.

The leaves of the elder, willow, and sloe are shown in Figs. 75, 76, and 77, in order that they may be compared with the tea leaf and with each other; the differences will be seen to be slight.

The leaf of the *elder* is seen to differ in the following respects: Its shape is ovate; the margin is sharply dentated and the apex

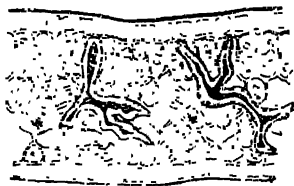



FIG. 80.—SECTION OF A TEA LEAF, SHOWING IDIOBLASTS. (× 150.)

is pointed; the leaf is, moreover, seen to be asymmetrical, due to the fact that one lateral half of it is attached lower down the midrib than the other.

The leaf of the *willow* differs in the following respects: It is elliptico-lanceolate in shape, with a pointed apex, and its contour presents shallow serrations.

The leaf of the *sloe* is in shape similar to that of the willow,

and the margin is slightly more deeply serrated than that of the tea leaf.

"Lie-tea" is the name given to a mixture of tea-dust with other leaves, clay, sand, etc., and made up into small masses with gum and starch.

The employment of used leaves is a more difficult matter to detect, as they may be prepared to resemble those which are unused. By uniformly colouring or "facing" them—in the case of green teas by indigo, aniline dyes, a mixture of Prussian blue, turmeric, and sulphate of lime, and in the case of black teas with blacklead—their appearance has been made to closely resemble that of unused leaves; but this "facing" is now rarely, if ever, done. Old leaves have been re-rolled and worked up with sand and gum; the sand or quartz serves the purpose of furnishing the stiffness of the natural unexhausted leaf when dried, and the gum, while also aiding in this, imparts a gloss to the otherwise dull-looking leaf.

The task of detecting leaves which have been previously exhausted and not subsequently made up is wellnigh impossible to achieve when these are only added in reasonable quantity; for genuine samples of tea vary so much as to the relative amounts of their constituents that some samples may have been partially exhausted and yet yield more extract to boiling water than other very poor but genuine samples.

Most will probably be learnt of this form of adulteration by the analysis of the ash of the leaves. Bell found that in average samples of genuine teas (dried at 100° C.) this never reached 8 per cent., and was generally from 4·7 to 6·2 per cent.; and the Society of Public Analysts has advocated 8 per cent. as the limit. The ash soluble in water does not fall below 3 per cent. by weight of tea, or 40 per cent. of the ash. It follows of necessity that a sample of tea containing much exhausted leaf will show a reduction below 3 per cent. of soluble ash.

The ash should be estimated by taking 10 grammes of tea, gently incinerating this in a platinum dish, and weighing the mineral residue, which is generally grey or greenish in colour. In "faced" teas the ash is sometimes 10 per cent., and in "lie-tea" it may amount to 30 per cent.; whereas in exhausted tea it is rarely above 0·8 per cent. The ash should then be treated with boiling water and the whole filtered through a small Swedish filter-paper; the insoluble part must next be thoroughly washed

on the filter, re-ignited, and weighed; the difference between the weight obtained and that of the total ash will represent soluble ash. If the insoluble residue is boiled with dilute hydrochloric acid, that which still remains insoluble (consisting mostly of sand and quartz) should not much exceed 1 per cent.

The *estimation of thein* is also of value where the use of exhausted leaves is suspected. Five grammes of the finely powdered and dried tea are three times extracted with about 300 c.c. of boiling water in a flask fitted to a reflux condenser, each extraction occupying two hours; the united extracts are then precipitated with neutral lead acetate, and the liquid again boiled for ten minutes and filtered; the filtrate is freed from lead by sulphuretted hydrogen and evaporated to dryness on the water-bath with freshly-ignited magnesia and clean coarse sand; the dry residue (finely powdered) is then thoroughly extracted with chloroform in Soxhlet's apparatus. The residue, after evaporating the chloroform extract, is boiled with water, filtered, and the filtrate evaporated down and dried at a temperature not exceeding 100° C. and weighed. Under the microscope the thein appears as long, white, silky needles.

It is of great importance that the preliminary extraction be made with the greatest thoroughness, on account of the obstinacy with which a little of the alkaloid is apt to be retained by the vegetable tissue.

Dvorkovitch, when estimating thein in tea, washes the extract with petroleum ether, in order to remove the oil and any traces of the brown substance found in tea.

A simple micro-chemical method for the detection of thein or caffein, which is rapid and requires only a small amount of the original material, is to place a tea leaf (or a similar quantity of the substance to be examined) on a watch-glass covered with another watch-glass of similar size, and to heat for five minutes over a small flame, the glasses being supported on a wire gauze about 7 centimetres above the source of heat. The upper cover-glass will then be found to show numerous droplets of condensed liquid; in ten minutes fine needle-shaped crystals will appear, and after fifteen minutes a good crop of these will be shown on microscopical examination. Exhausted infused tea leaves show no sign of such crystalline sublimate. Coffee berries and the leaves of the coffee-plant, kola-nuts, guarana, and maté, all give a characteristic sublimate of caffein when treated in this

manner. By applying cold water to the outside of the upper cover-glass, the appearance of the crystals may be materially hastened. That the sublimate consists of caffein may be proved by the usual chemical tests. When, for instance, caffein is treated with bromine water (avoiding excess) and the liquid evaporated to dryness over the water-bath, a yellowish residue is left, which becomes crimson-red on further heating, and is turned purple by ammonia. On adding caustic soda, a complete and instant discoloration occurs.

Sodium carbonate and borax have been added to deepen the colour of the infusion.

The tannin may be roughly estimated from the infusion of a weighed quantity of tea, from which it may be precipitated by gelatine; about 40 per cent. of the precipitate, when collected and dried, will consist of tannin.

In the *Lancet* laboratory it is found that good and consistent results are obtained by taking 20 c.c. of the fresh infusion, adding slight excess of bicarbonate of soda, and running in $\frac{N}{10}$ iodine solution until some petroleum ether (shaken after each addition with the mixture) shows a slight excess of iodine present by the well-known violet colour. The amount of tannin is each c.c. of $\frac{N}{10}$ iodine used multiplied by 0·0021.

INFANTS' FOODS.

There are many proprietary articles upon the market which have a large sale as "Infants' Foods," and they vary considerably in their value as nutrients. No standard or declaration of composition is demanded. Condensed milk has perhaps the largest sale (*vide* pp: 245-247). There is no legal limit fixed as to the amount of milk constituents which such a preparation should contain, but considering the dilutions generally recommended for the purposes of infant feeding, the fat ought not to be less than 10, nor the non-fatty milk solids less than 25·5 per cent.

It is of importance that no unchanged starch should enter into the composition of foods for infants under seven months, for the reason that they are incapable of properly digesting it. The starch is therefore often partly or wholly transformed into dextrine or malt sugar. The presence of starch may be best ascertained by the microscope, especially if a drop of iodine solution is allowed to run under the cover-glass. The nature of the starch

employed may also be detected by the microscopic examination. A quantitative estimation may be made by separating the sugar and other soluble matter from the preparation by cold water, and drying the residue (containing starch) by prolonged heating in a water-bath; an aliquot part (about 0·5 gramme) of this is then inverted, and the amount of starch calculated by multiplying the amount of invert sugar (estimated by Fehling's method, *vide* pp. 337-339) by 0·9.

There should be phosphates in these preparations. They may be dissolved out from the ash by nitric acid, and estimated by the method employed in water analysis. No chemical preservatives should be present.

The proteins may be estimated by Kjeldahl's process on 0·5 gramme of the material, and the total nitrogen estimated must be multiplied by 6·25.

The protein material generally falls between 8 to 14 per cent.; the fat from 1 to 12 per cent. (often it does not exceed 2 per cent.); the carbohydrates from 70 to 82 per cent.; and the mineral ash from 1 to 3 per cent.

It will be seen, therefore, that many infant foods are deficient in fat, but contain carbohydrate and protein in excess.

Dr. F. J. H. Coutts, in a Report to the Local Government Board, 1914, groups the proprietary infants' foods (apart from humanized, condensed, and dried milk) in the following five broad classes:

1. Foods with a basis of dried cow's milk, but mixed with flour.*

2. Foods consisting mainly of flours,* the starch of which is practically unaltered, or altered only by heating.

3. Foods consisting mainly of flours* mixed with a proportion of malt flour or malt extract, but containing much unaltered starch which is not converted during the process of preparing the food for infants in accordance with the directions given on the package.

4. Foods containing flours,* but also containing active diastase or pancreatic ferment, so that if the food is carefully prepared according to the directions on the package the starch is appreciably altered.

5. Foods manufactured from flours* the starch of which has

* The word "flour" is used here to include not only the true cereals, but other starch-containing vegetable substances—*e.g.*, flour from leguminous seeds, arrowroot, banana, etc.

been mainly or partially converted into soluble products during the course of manufacture.

In the same Report (1914) Mr. Julian L. Baker, F.I.C., cites the following analysis of the three principal ingredients which, either alone or blended, enter into the composition of most of these foods—viz., wheaten flour, dried milk, and dried malt extract. The last mentioned was made by mashing a barley malt with flour at a temperature of 60° C., filtering, boiling, and evaporating the liquid to dryness. This product was entirely soluble in water.

	Flour.	Dried Milk.	Malt Extract.
	Per Cent.	Per Cent.	Per Cent
Starch	70·4	Nil	Nil
Hydrolyzed starch products ..	Nil	Nil	90·0
Reducing sugars as dextrose (other than lactose)	1·8	Nil	—
Lactose	Nil	39·0	Nil
Cane-sugar	1·3	Nil	2·5
Fat	1·1	28·7	Trace
Proteins (N × 6·25)	11·0	23·8	4·1
Water	13·1	1·4	1·5
Mineral matter	0·4	5·8	1·5
Cellulose	1·5	Nil	Nil
	100·6	98·7	99·6

CHAPTER XIII

PRESERVED AND TINNED PROVISIONS

THE preservation of sterilized food in tins or cans is a valuable means of saving a large amount of food, and it thus cheapens living. Given that wholesome food is always used, and the process of canning and sterilization is efficiently conducted, the material remains good for a long period; but the *exclusive* use of canned meats may lead to scurvy.

Articles of food, when thus preserved, may be unwholesome from the following causes:

1. Changes in the food itself, owing to the development of ptomaines or toxins—these may be present prior to canning, or they may develop subsequent to canning, either before the tin is opened, or very shortly afterwards.

2. The addition of harmful chemical antiseptics to preserve the contents.

3. The addition of harmful substances employed as colouring agents.

4. Impurities yielded by "tins," or the solder used in their manufacture. By the action of the juices upon the tins, tin, lead, or even arsenic, may be taken up from the tins and solder. It is the vegetable acids naturally in the food, those that are formed during fermentation of vegetable matter, or agents which are added for preservative purposes (vinegar and oil), which act upon the metals; and this action may be increased by the galvanism which is sometimes set up between the metals present. Tinned asparagus, tomatoes, pears, cherries, plums, and apricots are especially liable to take up metals.

The contents of the tins are hermetically sealed down by solder at a high temperature, and the partial vacuum thus created in the tins is evidenced by their tops and bottoms being slightly depressed from the outside; should, however, there be any flaw

in the tins, or a solder seal be imperfectly applied, or should the heating process be but partially performed, then the contents may go bad, and in the latter case, owing to the accumulation of the gases of putrefaction, the tops and bottoms of the tins become quite flat, and later on convex outwards, and the tin when struck may give out a hollow or drumlike sound. It is not difficult, therefore, in some cases to detect, before opening them, those tins in which the contents are bad; and a "blown" tin is evidence of advanced decomposition. Beveridge accounts for the circumstance that tins exposed to unduly high temperatures, subsequent to canning, sometimes become "blown" by the fact that the spores of *Bacillus cadaveris sporogenes*, which are very resistant to heat, may not develop at low temperatures, but rapidly do so at temperatures of about 37° C. He therefore recommends that sample tins should be examined after incubation at 37° C. for a fortnight.

Minor degrees of gas-formation may best be detected by perforating the tins under water, when small bubbles of gas will be seen to rise. Viry maintains that putrefactive changes in canned foods *may* take place without the formation of any gas.

The bulging of tins, though generally produced by the formation of the gases of putrefaction, may be localized and due to rough treatment, whereby they are dented and the contents displaced. The condition may also be due to the freezing of tins containing liquid or semi-liquid food (as in cold storage), the tins bulging from the expansion of the frozen liquid.

It seems that bulging may also, in exceptional cases, be caused by gas produced by electrolytic action between the metal of which the cans are composed and acids in the contents. (This has occurred in condensed milk.)

As blown tins have been punctured to allow of the escape of the gases produced by putrefaction, and then resterilized and resoldered, it has been said that cans showing two solder points are suspicious; but in the event of such a fraudulent practice it is generally easier to melt the solder over the original blow-hole, and then resolder, than to make a second hole and solder it, and some firms invariably make a second blow-hole (especially in flat tins). An accidental splash of solder may simulate a second blow-hole.

In the process of canning, after the top has been soldered on and the small hole in the lid closed with solder, the can is either

placed in a steam retort at 115° C. for one or two hours, or in a boiling solution of calcium chloride (105° C.) for one or two hours, or in ordinary boiling water for four hours. The contents of the tin expand and the sides and ends slightly bulge. The can is then taken out of the apparatus, and the prick-hole is unsoldered by the application of a hot iron, when about 1 cubic inch of heated air escapes. The hole is soldered up afresh and the can put back again into the water-bath or steaming apparatus for another hour, and the sterilizing and cooking process completed. For blowing purposes during the bathing or steaming processes, instead of taking the trouble to expose and unsolder the prick-hole, some firms prefer making a second hole either at the side or at the other end of the can.

With condensed milk and with jams and syrups it is not absolutely necessary to have blow-holes, and in some brands none are to be found. These materials are already cooked, and are poured hot into the cans, which have concave ends when the contents are cooled down. The sugar acts as the preservative.

Nothing short of opening some of the cans will enable one to express a safe opinion with reference to the contents. From 2 to 5 per cent. should be opened, and the contents in some cases examined chemically and bacteriologically.

The writer has made many tests of canned nitrogenous food, both for final and intermediary products of decomposition—including ammonia, sulphuretted hydrogen, carbonic acid, basic degradation products of proteins, ptomaines, phenoloid bodies, etc. The results were variable and inconclusive when tests upon exposed material were compared with those upon the contents of the freshly-opened control tins. Certain advocated tests (such as the rod dipped either in Nessler's reagent, or in a mixture of hydrochloric acid, chloroform, and ether, in order to detect ammonia) were found to be practically useless; and, in short, no chemical test was found of value as an indication of definite decomposition changes before the stage was reached when such changes were equally apparent in the changed physical characters of the material. Earlier chemical evidence of change by the distillation of suitable solvent agents allowed to act upon the canned material furnished no definite results which would serve as a safe guide to practice.

Electro-conductivity tests for early decomposition changes also proved of little practical value.

A careful examination of the article (always aided by comparing the contents of an obviously sound tin with those of a suspected tin) will generally serve to detect the following physical signs of early decomposition changes, and such changes are no better indicated by difficult and tedious chemical and other physical tests:

1. *Odour.*—This is more or less changed. The odour would generally be described as one of "staleness"; but, however it is defined, the observer is generally able to appreciate some change. It is well to place any questionable material in a clean, odourless bottle, filled up to the neck with a little strong solution of caustic potash, stoppered with a ground-glass stopper, and placed in the hot incubator for an hour. A control of obviously fresh material is similarly dealt with. At the end of the hour any faint difference in odour generally becomes quite apparent. An offensive odour is generally a sign of very advanced decomposition.

2. *Firmness or Resistance to Pressure.*—Often a fairly early sign of decomposition is an appreciable loss of resistance when some of the article is pressed and broken up between the fingers and thumb. The article is slightly softer or more friable, and the contents of a tin are generally more easily turned out.

3. *Colour.*—A fading or change of natural colour is another useful sign of decomposition when it is associated with changes 1 and 2. When the colour changes it generally turns to a brownish tint. While the solid matter may show no such changes, any liquid matter present may do so, in which case the latter may show an increased opacity. The tin coating may also be slightly discoloured in places.

The reaction, which in the case of meats is usually acid, may be expressed in terms of lactic acid (1 c.c. of decinormal soda =0·009 gramme of lactic acid). The mineral ash is never white in the case of meat, and its reaction should be alkaline. In estimating the fat, 2 grammes of the dried material are extracted in Soxhlet's apparatus. The protein material is calculated from the total nitrogen of 1 gramme obtained by Kjeldahl's process. In order to obtain a satisfactory sample of the contents, the whole of them should be twice passed through a fine mincing machine, and then ground up in a mortar.

THE AVERAGE PERCENTAGE COMPOSITION OF TINNED MEATS (BEVERIDGE).

	Water.	Ash.	Protein.	Fat.
	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Corned beef	51·05	3·56	28·72	17·74
Roast beef	58·23	3·22	26·75	12·90
Corned mutton	43·05	2·39	26·34	28·12
Roast mutton	46·22	1·52	26·50	25·74

It is very important that the tin or can in which these food-stuffs are placed should be satisfactory. The coating of tin should be properly done, so that no flaws are perceptible with a magnifying-glass, as otherwise the iron beneath will rust through. Potassium ferricyanide solution may be employed to demonstrate (by its bright blue precipitate with the exposed iron) the presence of pin-hole flaws. The tin used for coating ought not to contain more than 1 per cent. of lead, and "terne plate" (two of tin to one of lead) should be prohibited. The solder employed ought not to contain more than 10 per cent. of lead, and should be confined to the outside of the can. Cans containing very acid juice—namely, vinegar, plum, and asparagus juices—should be lacquered inside.

The soldering should be performed so that it is impossible for it to reach any internal surface of the can; and in the majority of tins as now made this is only possible by the metal or soldering fluid being accessible to the contents of the can in the operation of closing the vent-hole. Many of the better-class manufacturers provide against this accident by means of a solder-trap consisting of a small, cup-shaped piece of tin attached immediately beneath the vent-hole.

Poisonous symptoms have been traced to the presence of **tin** in preserved foods. Where the metal is eroded, which is commonly at spots where it has been in contact with fat, the cause of the erosion is due to the formation of basic stannous chloride. A tin sulphide is also sometimes formed by the action of decomposing albuminous matters. Varnished tins are used by some French manufacturers, and lead has sometimes been found in this varnish. Tin is but slightly soluble in acetic and other organic acids, and in the absence of oxygen, and its absorption is very slight in the gastro-intestinal tract. Instances of poisoning have,

however, been recorded where tin to the amount of from 15 to 20 grains to the pound was found in the food. The metal has little tendency to accumulate in the system. But the organic acids of preserved fruit, tinned tomatoes, meat extracts and essences are often capable of dissolving many grains per pound, and the amount taken up increases with the age of canning. There is little evidence of danger from the amounts of this metal which are generally to be found in canned foods; but it must be borne in mind that obscure ailments, possibly associated with the ingestion of such foods, are difficult to refer to their specific causes.

The erosion of the tin may by exposing the iron enable an electrolytic action to lead to a further solution of tin.

In a Report of the Inspector of Foods issued by the Local Government Board in 1908, the special attention of sanitary officers and public analysts is directed to canned food of more than one or two years old; and it is stated that if 2 grains of tin per pound are found, it may be taken that the food has become potentially deleterious to health. In the estimation of tin in such material, Dr. Schryver destroys 50 grammes of the sample by the Kjeldahl process; he then dilutes to 600 c.c., treats with sulphuretted hydrogen, and allows to stand overnight. The precipitate of tin sulphide is collected, washed, and dissolved in a small quantity of hot 10 per cent. sodium hydroxide solution, and the sulphide reprecipitated with glacial acetic acid (this removes silica, etc.). The sulphide is then collected on a filter, washed, dried, oxidized, and the tin weighed as oxide.

A delicate test for the presence of tin has been found in dinitrodiphenylaminesulphoxide. Stannous chloride in the presence of an excess of hydrochloric acid furnishes with this reagent a brilliant violet colouring matter.

Tin-foil has been shown to be capable of furnishing **lead** to sweets.

The unscientific use of lead in the composition of glaze for enamelled saucepans and dishes used in cooking and in the storing of food has led to several accidents. The fat or acidity of food may dissolve some of the lead in the glaze. Glazed vessels intended for domestic purposes should not yield any lead when a 4 per cent. solution of acetic acid is boiled in them for half an hour. This test is by no means too stringent. Glass vessels are the most hygienic receptacles in which food may be stored; but their use entails a disadvantage from their liability to break.

It is not necessary to repeat here the means of testing for the various metals, since their characteristic reactions have been seen in treating of Water. The presence of **copper,** however, can often be roughly demonstrated by allowing a piece of steel, such as a knife-blade, to lie in the liquid (acidified with sulphuric acid) for a short time, and noting the appearance of a bronze coloration upon it. To estimate the amount, extract the copper from the ash of a considerable amount of the material; the grey ash should be treated with concentrated sulphuric acid, and then the residual carbon burnt off in a muffle. The ash should next be extracted with nitro-hydrochloric acid; excess of ammonia (1 : 3) added; then filter, and wash with dilute ammonia. Make up the filtrate to 50 c.c., and transfer to a Nessler glass. Then match the blue tint by taking a second Nessler glass, adding ammonia in a similar quantity to that contained in the other Nessler glass, filling up the cylinder to the 50 c.c. mark with distilled water, and running in the necessary amount of a standard copper solution (1 c.c.= 0·1 milligramme of Cu).

Aluminium is now becoming cheaper, and the metal is extremely well adapted for making into "tins," pots, canteens, etc., on account of its lightness, ductility, and malleability, and owing to the fact that its bright appearance is very little affected by damp. Alcohol can dissolve up the metal in a slight degree, and acids—even acetic and lactic—have a similar power, though more marked. It is probable, however, that even in the latter case there is not sufficient dissolved to give rise to symptoms of poisoning under the common conditions of food-potting, for the metal is not a very poisonous one. It also appears that for ordinary culinary purposes the use of aluminium cooking vessels is not attended by any risks to health.

Doubtless many of the cases of poisoning attributed to metals have really been the results of toxins, etc., in bad food.

Preserved vegetables have commonly been found to be coloured (green) by **copper sulphate.** The coloration is attributed to the formation of a copper salt by an acid derived from phyllocyanin (a derivative of chlorophyll), which body is very inert and insoluble in hydrochloric acid. Any excess of copper combines with the proteid matter to form copper leguminate, which is practically useless for colouring purposes.

The general practice is as follows: The peas are treated with a solution of cupric sulphate; this is almost immediately poured

off, and the peas are subsequently well washed with water. They are next boiled in their tins, and then soldered up. Some authorities have pronounced in favour of the harmlessness of this employment of cupric sulphate when the amount used does not exceed 2 grains (about $\frac{1}{2}$ grain Cu) to the pound of peas, beans, spinach, etc.; but the Departmental Committee appointed in 1899 recommended that the use of copper for colouring food should be prohibited (as in Germany and Austria). In small quantities copper is found naturally in certain foods (notably oysters), and Lehmann assumes that nearly $\frac{1}{3}$ grain may thus be taken in daily.

Sulphate of copper acts as a powerful astringent upon the lining membranes of the stomach and intestines. It also interferes with digestion by reason of its powers of inhibiting the digestive ferments, even when present in very small quantity. In large doses, or in smaller doses frequently repeated, it is an irritant poison, occasioning symptoms closely resembling those due to lead-poisoning.

W. Ogilvie and M'Lean Wilson separated the copper colouring matter from peas; and the former, by experiments upon mice and also upon his own body, showed that the organic salts of copper thus obtained were absorbed by the alimentary canal of man and mice, that they tend to accumulate in the liver, and are mainly excreted in the urine and to a slight extent by the salivary glands. The copper compound is soluble in artificial gastric juice, made by adding Benger's liquor pepticus to 0·2 per cent. HCl in the proportion of 1 to 5, the whole being kept at a temperature of 40° C. for several hours. F. A. Cripps has shown that 90 per cent. of the copper salts are thus dissolved in two hours. The old theory that the copper formed with the legumen of peas, spinach, etc., a compound which is quite insoluble by the gastro-intestinal secretions, is therefore erroneous.

It is by no means uncommon to find **chemical preservatives** other than salt and saltpetre in meat foods packed in cans or glass. Of these preservatives boron compounds constitute about three-fourths of the whole, and sulphite preservatives constitute almost the whole of the remainder. Their presence points to the probability that they were employed to overcome undesirable conditions either in the meat prior to canning and occasioned by uncleanly processes or insufficient sterilization during its manufacture. Sulphurous acid and sulphites are sometimes used as a spray or in a pickling fluid either to keep meat

fresh or to revivify it after it has become stale; the sulphurous acid employed diminishes in amount on keeping. Hams and bacon are sometimes packed between thin layers of powdered borax when they arrive in this country from abroad; and Richards and others have shown that boric acid which has been in contact with hams for a period of three or four weeks penetrates their substance to a very considerable degree, reaching even the most remote parts of the muscular substance, but penetrating only to a very slight extent into the fat.

Dr. A. W. J. MacFadden states in a report to the Local Government Board (1908) that amounts of boric acid varying from 2·6 to 13·5 grains per pound of minced and mixed ham may result from mere contact with the preservative in the packing-cases. He furthermore expresses the view that certain specified chemical preservatives should not be used in the preparation of *canned meats*, and that in any schedule of prohibited preservatives boron compounds, sulphites and preparations of sulphurous acids, benzoic acid, and formalin should be included.

The boric acid contained in these foods may vary from a fraction of a grain per pound to 50 grains, or even more, the amount depending on whether the preservative has been employed only as a packing material or has, in addition, been used in the curing of the meat.

In the making of *sausages* and other minced meat preparations there is a temptation to obtain raw materials cheaply by purchasing the material at a time when fresh meats have reached the limit of their keeping powers, and it seems a fair assumption that where this material is sold packed in cans or glass, and chemical preservatives are found, either the material or the canning process was faulty, as in the alternative case their employment is quite unnecessary.

Dr. A. W. J. MacFadden, in reporting upon this subject to the Local Government Board, recommends (1908) that if boron preparations are used for the preservation of sausages, etc., a limit of ¼ per cent. of boric acid (17·5 grains per pound) would probably be ample to meet the different trade requirements, and even then it should be considered as to whether a notification of the presence of the preservatives should not be given to the purchaser.

In the cheaper brands of potted meats a certain quantity of rice-flour or other cereal, varying from 10 to 50 per cent., or even more, is generally mixed with the meat.

CHAPTER XIV

CHEMICAL ANTISEPTICS AND COLOURING AGENTS IN FOOD

THE employment of chemical agents which will prevent the development of the micro-organisms concerned in putrefaction, and termed "antiseptics," is extensively practised. The increasing use of these substances for the preservation of articles of food, and the necessity of keeping a rigid control over this practice in the interest of public health, has rendered the detection and estimation of such substances a very important matter. If any such addition to food was illegal, unless and until its harmlessness had first been established, the public would be better protected than at present. The antiseptics most commonly employed are: Borax and boric acid, salicylates, benzoates, formaldehyde (a 38 to 40 per cent. solution of which in water containing a small quantity of methyl alcohol is known to the trade as "formalin"), sulphurous acid, bisulphite of calcium, sodium chloride, and vinegar; but saltpetre, sodium fluoride and silicofluorides, spirits of wine, and sulphate of copper, have all been employed. Salicylic acid is depressing, it is liable to be cumulative in action, and it has an irritant effect upon the kidneys; benzoic acid is irritating; sulphurous acid is a gastric irritant; and formaldehyde has a strong tendency to combine with proteids and to harden them and reduce their digestibility. Being antiseptics, and therefore inimical to the life of the organisms that cause putrefaction, they *must* exercise a retarding effect upon the activity of the enzymes concerned in ordinary digestion.* Halliburton has shown that as little as 0·05 per cent. of formaldehyde delays gastric digestion; and outbreaks of dermatitis have been attributed to formalin in milk.

It is certain that boric acid, salicylic acid, formic aldehyde, etc., are foreign to the animal body, and their presence must

* These enzymes or digestive ferments are non-living, unorganized nitrogenous bodies, which can transform other organic bodies without exhausting themselves.

necessitate a departure from the normal chemistry of life. They, moreover, facilitate an uncleanly, slovenly treatment of food, and render it possible to preserve articles in incipient decomposition for some time with every appearance of freshness.

There is almost a consensus of opinion in the medical profession that these agents, as often employed, may be injurious to health. *Boric acid* and its salts are probably the least harmful, and doubtless the average adult could take a few grains daily of boric acid with little, if any, ill consequences; but these antiseptics are frequently added to foodstuffs by ignorant persons in amounts far exceeding those necessary to effect the object desired. Thus, nearly 20 grains of boric acid have been found in a pint of milk, doubtless from the fact that the milk had been dosed by the farmer in the first place, and subsequently by dairymen; 52 grains per pound have been found in potted meat, and 150 grains per pound in cooked tripe imported from America. The British Pharmacopœia includes boric acid and borax amongst its drugs, and gives the dose of the former as from 5 to 15 grains, and of the latter from 5 to 20 grains, for an adult. It is therefore regarded as a drug capable of producing physiological effects upon human adults, even in a dose of 5 grains.

Boric acid is to some extent cumulative for a few days, after which the daily amount eliminated balances the amount consumed. Eighty per cent. of the boric acid and borax ingested is eliminated by the kidneys; and it must certainly be very harmful to those suffering from kidney disease. It may be mentioned, further, that a special harmfulness to infants is indicated by the fact that borax retards to some extent the coagulation of milk by the rennet ferment of the gastric juice, and that as little as one part per 1,000 completely inhibits the activity of this ferment (Halliburton). The same authority has shown that when boric acid is added to milk it precipitates the lime salts (which are required by infants for the proper formation of bone and teeth) as the insoluble borate of calcium.

Individual susceptibility to the drug varies considerably; but there is a considerable amount of evidence, of an experimental nature upon human beings, that boric acid administered in doses of 15 grains and upwards for several days may cause headache, loss of appetite, vomiting, diarrhœa, depression, and certain skin eruptions. The writer has on two separate occasions seriously disturbed his digestion by taking 20 grains of boric

acid daily, with food, for only three days. Doubtless injury to health would follow upon the use of even a few grains daily when administered to babies in milk. Experiments upon human beings have produced conflicting results, but the positive evidence of harm obtained by many far outweighs in importance the negative results which have been obtained by others. The latter experiments have almost all of them been upon non-susceptible *adults* and for *brief periods* ; and there is no evidence whatever which points to the harmlessness of these drugs upon infants under one year of age when the milk (which is almost the sole article of their diet) is dosed with boric acid for long periods, or upon adults with weak stomachs or kidneys who take 10 to 15 grains several times a week in food.

Dr. Wiley's experiments (1905), carried out at the instance of the United States Department of Agriculture, were feeding experiments, extending over twenty-eight weeks, upon twelve healthy young men. They demonstrated that when only 8 grains of boric acid (or its equivalent in borax) is daily taken with food for seven weeks or over, in some instances impairment of appetite with gastro-intestinal disturbance and headache resulted, and that these sensations disappeared when the preservative was withdrawn.

Dr. Wiley similarly experimented upon the effect of *salicylic acid* and the salicylates. For thirty days salicylic acid was given in doses gradually increasing from 3 to 30 grains. A loss of weight was observed in almost all cases. A large part of the salicylic acid was excreted unchanged by the urine, and in four men a tendency was shown to albuminuria. He found that, when added to food even in small quantities, a depressing and harmful effect upon health and digestion and a disturbance of the general metabolic activities of the body were manifested.

In 1907 experiments were also conducted to study the effects of the administration of *sulphurous acid* and sulphites with food. The preservative was administered in two forms, sodium sulphite being administered in capsules to one-half the men, while sulphurous acid was added to the drinking-water of the others. The average daily consumption for twenty days was 0·392 to 0·628 gramme of sodium sulphite, and 0·213 to 0·343 gramme of sulphur dioxide. The medical and clinical data, in Dr. Wiley's opinion, showed that sulphurous acid and its salts in the free state produce harmful effects, the metabolic functions being

disturbed and the health (particularly the digestion) injuriously affected. In the great majority of cases headache, sensations of dizziness, and occasional nausea, indigestion, pains in the stomach, exhaustion and weakness ensued. In some cases palpitation of the heart and other unfavourable symptoms were noticed. It was also observed that there was a marked tendency to albuminuria and a reduction in the quantity of hæmoglobin and in the number of red and white blood-corpuscles (particularly of the latter).

Still more recently the same observer has undertaken an investigation into the effect of *formic aldehyde* upon digestion and the health. A daily dose of formaldehyde, varying from 100 to 200 milligrammes, was given in milk to twelve healthy men. No marked symptoms were noticed during the first ten days, but then headache and pain in the stomach and intestines became general, in many cases producing cramps, and in a few cases nausea and vomiting. A burning sensation in the throat was reported in the majority of cases, and in four of the subjects a well-marked itching rash appeared. From a general study of all the data Dr. Wiley draws the conclusion that the admixture of formaldehyde with food is injurious to health, even in the case of healthy young men, and that therefore in the case of infants and children the deleterious effects would be more pronounced. The metabolic functions were disturbed in a notable way.

Dr. Wiley's experiments with benzoic acid and benzoates (1908) demonstrated that the prevalent view that these agents are the least harmful preservatives in food is a false one. The preservatives were added to the food and were given in daily doses of from 1 to 2½ grammes, the dose being increased gradually within these limits during a period extending over twenty days, the total amount given during the entire period ranging in the case of the different individuals from 13½ to 35 grammes. There was but little difference noted in the effects of benzoic acid and sodium benzoate. Nausea was produced in nine cases and headache in eight, vomiting supervened in three, while seven of the subjects complained of weakenss and of burning and irritating sensations in the œsophagus. Hunger was increased in three cases, and indigestion was especially noted five times. The men who took benzoic acid lost about two pounds in weight, but in the case of those who took sodium benzoate

a smaller loss took place. Benzoic acid is partly eliminated as hippuric acid, but there is a tendency to retain benzoic acid in the body for a notable length of time. An examination of the fæces indicated a tendency on the part of the preservative to diminish assimilation. A marked increase was noted in the total solids excreted in the urine, although the volume was slightly diminished, and the presence of increased numbers of epithelial cells, mucous strands, and other microscopical bodies afforded further evidence of the greater activity of the kidneys induced by the ingestion of these preservatives. While the administration of both benzoic acid and its sodium salt is undoubtedly harmful, the injurious action is more rapidly noticed in the case of the acid than of the salt. The total harmful effect, however, is practically the same in both. Dr. Wiley considers that the final conclusion to be drawn from the data is that in the interests of health both benzoic acid and its sodium salt should be excluded from food products, as both these substances, when added to foods, are injurious to health.

The use of chemical antiseptics in food is not necessary. Even dairy produce is brought from Denmark, etc., without such addition, and no difficulty is experienced by milk vendors in this country and abroad in selling to the consumer even in the summer months, milk in a perfectly fresh state to which no chemicals have been added.

The presence of chemical preservatives in canned articles which have been sterilized by heat indicate that the addition was made, prior to canning, to check decomposition. With good material their use is entirely unnecessary, and their presence is not expected by the purchaser.

But it is not only in one article of a meal that one may find chemical preservatives. At breakfast they may be consumed in milk, butter, ham or bacon, and jam. Thus, though the quantity of boric acid in any one article might not be sufficient to affect health, the aggregate amount of the drug taken in the various articles of the meal may suffice; and impaired digestion and nutrition or more serious disturbance may result.

There is but little direct evidence in the community of boric acid poisoning from food, because the symptoms are not characteristic, and may be due to many causes, and in any particular case their origin in chemical preservatives is not even suspected. It may be maintained that such evidence would not be

lacking if the employment of these preservatives in food had in every case to be declared to the public.

Those who advocate the use of chemical antiseptics maintain that: (1) They obviate waste, inasmuch as without their use a large amount of food would go bad and have to be destroyed. Milk, it is said, if delivered in a fresh state, will not keep in the homes of the poor without antiseptics. (2) If not used, the injury resulting from the consumption of fermenting and decomposing food would exceed that from the ingestion of chemical antiseptics. (3) The experiments hitherto made (more especially upon the lower animals) furnish quite as much evidence of the harmlessness of these agents as of their harmfulness.

None of these arguments are of much weight. Arguments 1 and 2 are belied by the practical experience of many countries where chemical preservatives, even in milk and butter, are disallowed. There is no evidence in support of the argument that material sold as fresh, such as milk and fresh sausages, goes bad in the homes of the poor. The poor quickly consume what they purchase; they do not buy to store. As to Argument 3, it is impossible to argue from the effects which feeding experiments may have upon the lower animals to those which may be produced upon very young or sick human beings.

Certainly, if the use of these chemical preservatives is to be sanctioned, the amounts should be limited by law; and the individual who is not desirous of continuously dosing himself with drugs should have a means of escape from them. Their presence, nature, and amount should be legibly set out upon a label, and their use ought most certainly to be prohibited in infants' and invalids' foods. It may be said, however, that even the safeguard of a label assumes an amount of knowledge and discrimination on the part of the purchaser which is unreasonable. In New South Wales (Public Health Act, 1902) the amounts permitted are as follows: Sulphurous acid, 1¾ grains; salicylic and benzoic acid, 1 grain; and boric acid, 10 grains—per pint of liquid or per pound of solid food.

The *Departmental Committee appointed in* 1899 to consider the subject of the use of antiseptics and colouring agents in food, in their Report (1901) made the following recommendations:

1. That the use of formaldehyde or formalin, or preparations thereof, in food or drinks be absolutely prohibited.

2. That salicylic acid be not used in a greater proportion than 1 grain per pint in liquid food, and 1 grain per pound in solid food; its presence in all cases to be declared.

3. That the use of any preservative or colouring matter whatever in milk offered for sale in the United Kingdom be constituted an offence under the Sale of Food and Drugs Act.

4. That the only preservative which it shall be lawful to use in cream be boric acid, or mixtures of boric acid and borax, and in amount not exceeding 0·25 per cent. (17·5 grains to the pound), expressed as boric acid, the amount of such preservative to be notified by a label upon the vessel.

5. That the only preservative which it shall be lawful to use in butter and margarine be boric acid or mixtures of boric acid and borax, to be used in proportions not exceeding 0·5 per cent. (35 grains to the pound), expressed as boric acid.

6. That in the case of all dietetic preparations intended for the use of invalids or infants chemical preservatives of all kinds be prohibited.

7. That the use of copper salts in the so-called "greening" of preserved foods be prohibited.

In a circular issued by the Local Government Board in 1906 it is stated that as regards formalin and boron preservatives the Board are advised that the presence in milk of formalin to an amount which is ascertained by examination *within three days of collecting the sample* to exceed 1 part in 40,000 (1 part in 100,000 of formic aldehyde) raises a strong presumption that the article has been rendered injurious to health, and that the purchaser has been prejudiced, in the above sense; and that a similar presumption is raised where boron preservatives are present in milk to an amount exceeding 57 parts of boric acid per 100,000 (40 grains per gallon, or 4 grains to the pound).

Experiments to test the effect of chemical antiseptics on health or digestion proceed on the following lines:

1. Solutions of starch are subjected to the action of the salivary and pancreatic ferments at appropriate temperatures, and a comparison is made of the amount of sugar formed before and after the addition of the preservatives. Similar experiments are also made on the peptic digestion of meat and the pancreatic digestion of casein. The length of time taken in the conversion before and after the addition of the antiseptic should also be compared.

2. The feeding of animals on food to which known quantities of the antiseptics have been added, and then either (*a*) weighing and examining the fæces in order to ascertain the amount of unassimilated nitrogenous and fatty matter; (*b*) noting the effect upon the body-weight of the animals from day to day; (*c*) observing the effect upon the intestinal tract (diarrhœa, sickness) or the general system (loss of appetite, discomfort, etc.).

3. Experimenters are sometimes prepared to experiment upon themselves, and then by making estimations of the nitrogen and fat in the food and excreta ascertain whether the albuminous matter and fat of the fæces increase in amount.

A means of food preservation which is open to no objections on the ground of public health is cold storage.

The heat and smoke produced by burning certain woods are used for preserving fish (herrings, haddocks, etc.), and the practice is proved by experience to be unobjectionable. The preservative effects of smoke appear to be dependent on traces of formic aldehyde, creosote, etc., in the smoke.

Salting is a very universal method of preserving meat, fish, eggs, olives, etc.; vinegar and sugar are also frequently used, and are equally unobjectionable. As decoloration results from salting meat, a little potassium nitrate (saltpetre) is commonly added to counteract this; and in small quantities it is unobjectionable.

Many methods depend upon the exclusion of air. Coatings of gelatine or glycerine, of collodion, and of paraffin wax have been employed with meat; salts which chemically combine with oxygen of the air, such as sulphite of lime, have been used; and oils, as in sardines, to exclude air.

The chemical preservatives to be specially sought for are: In meats (hams, bacon, sausage, oysters, shrimps, etc.)—boric acid and borax, sulphites, and salicylic acid. Boric acid and borax are preferred because they help to preserve the natural colour better than common salt, etc. In milk and milk products—boric acid, borax, formic aldehyde, occasionally benzoates, and often a mixture of boric acid and borax were employed before their addition was made illegal. In jams, jellies, mincemeat, and table delicacies—salicylic acid and benzoic acid or their salts, and occasionally boric acid. In cider, British wines, and fruit juices—salicylic acid and sulphites. In fermented beverages—salicylic acid, sulphites, fluorides, silico-fluorides, and boro-

fluorides. Saccharin may be present in beers, wines, and sweetened articles.

Salicylic acid is sparingly soluble in water and unpleasant to the taste; it is therefore mostly employed in highly flavoured articles, such as wines and jams.

If meat is packed in borax, the substance of the lean flesh is penetrated for some distance by the antiseptic; but there is little penetration into the fat.

Traces (less than a grain to the pound) of boron are to be found in nearly all fruits and vegetables.

Boric Acid and Borates.—Evaporate 100 c.c. of the milk, cider, wine, etc., to dryness, after rendering alkaline with caustic soda solution; incinerate; extract the ash with a little hydrochloric acid, filter, and evaporate the filtrate to dryness. Apply a very small quantity of diluted HCl to damp the ash; add a few drops of a fresh saturated turmeric solution, and evaporate to dryness. The dried residue is brownish-red; and a transient blue colour results, changing to a green, when a little water and alkali are added to the residue. Or, after adding just sufficient hydrochloric acid to the aqueous extract of the ash to furnish slight acidity, dip in a piece of turmeric paper, dry this at a gentle heat, when it turns a reddish colour, changing to a dark bluish-green on moistening with an alkali.

C. E. Cassal and H. Gerrans find that an intense magenta-red colour is produced on treating solutions containing boric acid with curcumin (or ordinary turmeric) and oxalic acid, and drying the mixture on the water-bath. The colour is different to that obtained by the application of the ordinary turmeric test for boric acid, and the reaction is far more delicate, extremely minute quantities of boric acid being easily detected. In applying the test for the detection of free or combined boric acid in milk and other food products, it is convenient as a rule to operate on an ash. The ash is treated with a few drops of (1) dilute hydrochloric acid, (2) saturated solution of oxalic acid, and (3) alcoholic solution of curcumin or turmeric; and the mixture is dried on the water-bath and taken up with a little alcohol. In cases where the amount of boric acid is very small, the substance the ash of which is to be operated upon should be made alkaline with a solution of barium hydroxide prior to evaporation and incineration.

All distillation, evaporation, or incineration methods result in

some loss of boric acid, and therefore lead to under-estimation. A. W. Stokes recommends the following method: Place 10 to 20 grammes of milk or cream in a tube, add four times the bulk of hot methylated spirit and 0·5 c.c. of normal sulphuric acid. This latter is used to turn out the boric acid from any of its compounds—borax, for instance. Shake vigorously, rotate in a centrifugal apparatus, or let settle for an hour. Filter through a dry filter-paper, pouring off the liquid only. To the residue in the tube add a little hot methylated spirit, shake, pour on to the filter, and wash with a little hot methylated spirit. The filtrate will, if kept hot, be quite clear, and it will now contain all the boric acid. Add 0·5 c.c. of strong phenolphthalein solution to the hot filtrate, and carefully titrate (while hot) with decinormal soda solution till there is a *slight* permanent pink colour. With a little care and experience it is quite possible not to overstep the addition of soda, for the pink colour produced in any case will be only slight. Neglect the number of c.c. of soda solution used, as their purpose is only to neutralize the liquid. Add to the hot solution half its present bulk of glycerine; the pink colour will disappear. Add from a burette decinormal soda solution till a pink colour reappears. Note only the number of c.c. used in this latter step, and from them calculate the quantity of boric acid present by multiplying the number of c.c. by 0·0062. This will give the total boric acid and borax in terms of H_3BO_3. In the case of butter, 10 to 20 grammes should be washed (after 0·5 c.c. of normal H_2SO_4 is added) in a separating funnel with three successive quantities of hot water, these washings being allowed to flow out into a small flask. The contents of this flask are to be titrated just as for milk or cream, first adding decinormal NaHO till a faint pink colour appears, then adding glycerine and titrating with the NaHO.

If boric acid has been added to milk, it cannot be detected as *free* boric acid after about two days, as by that time it combines with the lime-salts of the milk.

To test for boric acid in meat, mince the meat and warm with about an equal bulk of distilled water acidified with hydrochloric acid for half an hour; the liquid is then decanted, filtered, evaporated to dryness, the solid residue ashed, and the aqueous extract of the ash, slightly acidified with hydrochloric acid, is tested by turmeric paper.

The distillate obtained by boiling the minced meat would

also furnish evidence of traces of formic aldehyde (if such are present) if a drop of milk be added to the distillate and the mixture exposed to Hehner's test (see below).

For a close estimation of boric acid, 50 grammes of the meat are incinerated (complete combustion is not essential), transferred to a short-necked flask, and acidified with hydrochloric acid. A little solid calcium chloride is added, and the whole distilled in a current of methyl alcohol until the distillate no longer contains boric acid. If calcium chloride is not added, a considerable amount of boric acid will be retained in the distilling flask. The flask is connected with a condenser, and four or five portions of 20 c.c. each of methyl-alcohol are distilled (by means of a calcium chloride bath) into sodium hydroxide. The distillate is evaporated to dryness to expel the methyl alcohol, care being taken that it is distinctly alkaline; the residue is dissolved in 20 c.c. of water acidified with hydrochloric acid, heated to the boiling-point to expel carbonic acid, and the boric acid is then estimated in the manner described on the preceding page.

Formaldehyde.—By heating a solution containing this antiseptic small amounts may often be detected in the odour given off. In milk it is mostly or entirely gone in three days, and it is always greatly reduced in forty-eight hours.

Hehner's test is to float the milk on H_2SO_4 (90 to 94 per cent.) in a test-tube; a slight greenish tinge forms at the junction of the two liquids if formic aldehyde is absent, but a violet ring if present. The colour is permanent for three days. If milk be first diluted with an equal bulk of water, the delicacy of the test is increased.

Hehner states that the reaction depends upon the presence of casein, and that is the reason for adding a drop of milk to wine, vinegar, etc. The commercial acid should be employed, as the acid should contain a trace of ferric salt as impurity. The addition of a little beef-peptone increases the delicacy of the test. The test is unsatisfactory in the presence of hydrogen peroxide, but then positive results may be obtained after removing the H_2O_2 by means of reducing agents. A delicate means of testing is to examine the first portions of the distillate when the article is liquid (such as milk, wine, etc.).

To test wine or vinegar, add a drop of milk to the sample, and then pour the mixture on to H_2SO_4; a blue ring forms if formic aldehyde is present, but not so with only ordinary aldehyde.

To test butter, examine the aqueous liquor which separates when the butter is melted.

Dr. G. W. Monier-Williams reported (1912) to the Local Government Board upon the subject of a new preservative ("mystin") which had been found in milk. The substance consisted of a mixture of formaldehyde and sodium nitrite, and the effect of adding the sodium nitrite was to prevent the Hehner reaction from indicating the presence of formaldehyde. The "mystin" may, however, be detected by distilling over the formaldehyde from the milk, and by applying the Griess-Ilosvay test for nitrites.

The following test for formaldehyde is in use in the laboratory of the State Board of Health, Massachusetts: 10 c.c. of hydrochloric acid (specific gravity 1·2) are added to an equal amount of milk in a porcelain dish. A drop of dilute ferric chloride solution is added, and the mixture heated to just below the boiling-point and vigorously stirred. The presence of formalin is indicated by a violet coloration, varying in depth with the amount present.

An approximate estimation may be made (Liverseege) as follows: The reagent consists of a mixture of 100 c.c. of sulphuric acid and 2·5 c.c. of normal ferric chloride, which, as already indicated, causes the formation of a violet-blue ring when added to milk containing formaldehyde. Ten c.c. of the suspected sample are put into a 25 c.c. stoppered cylinder, and the reagent is added (1 c.c. at a time) until a violet colour appears and does not increase in intensity. By making experiments side by side with samples containing a definite proportion of formaldehyde, a fair idea as to the percentage may be obtained, as the more formaldehyde is present, the sooner the violet colour forms.

W. Scott Tebb suggests another useful method of making a very approximate colorimetric estimation of formic aldehyde. The method depends on the pink colour which develops when fuchsin decolorized with a saturated solution of sulphurous acid (Schiff's reagent) is added to the clear filtrate obtained after the separation (by means of acid) of casein and fat from diluted milk.

Fifty c.c. of the clear filtrate from the milk are poured into a Nessler glass, 5 c.c. of Schiff's reagent are added, and the mixture is allowed to stand for ten minutes. In estimating small traces of formaldehyde, which may readily be done if the Schiff's reagent is sufficiently sensitive, it is advisable to allow the liquid to stand

for a longer period—that is to say, for half an hour or even an hour. To estimate the exact percentage of the formaldehyde a number of standards must be prepared of known amounts of formalin added to milk. Each standard is treated by precipitation, filtration, etc., in precisely the same manner as the milk under examination. The Schiff's reagent is then added, and the colour of the sample in the Nessler glass is matched with the nearest standard. In ordinary milk adulteration from 0·002 to 0·01 per cent. of formaldehyde may be expected, and eight standards, containing 0·002 per cent., 0·01 per cent., and six intermediate percentages, should be made up.

Legler's method, as modified by A. G. Craig, is satisfactory. The requisites to the determination are a normal solution of sulphuric acid, an approximately normal solution of ammonia (the exact strength being immaterial), and a methyl-orange solution; 3-ounce bottles with smooth sides and close-fitting soft-rubber stoppers, and a boiler in which they may be immersed to the neck. Place 25 c.c. of the ammonia solution in each bottle, but to one-half of them add a sample of milk containing 0·5 gramme of formaldehyde. Stopper tightly, place the bottles in the boiler, fill with water to the neck, and boil for one hour. Cool slowly, and titrate carefully with sulphuric acid and methyl orange to the first indication of a colour change. From the differences between the readings the ammonia consumed in normal cubic centimetres may be calculated; every c.c.=0·0601 gramme of formaldehyde.

The errors in the Legler method do not counterbalance one another, the tendency being toward low results. A blank determination is necessary, and in the titration an accurate end-point is very important. Any acid present must be also accounted for.

Schuch tested several methods as to their suitability for the detection of formaldehyde in wines and in presence of acetaldehyde. The best process is that of Arnold and Mentzel. Three hundred c.c. of wine are distilled until 10 c.c. have passed over; then 5 c.c. are shaken with 1·5 c.c. of a solution of phenylhydrazine hydrochloride (1 : 50), and 4 drops of ferric chloride and 12 drops of sulphuric acid are added. In the presence of formaldehyde, a rose or dark red coloration is formed.

When meat is exposed to the vapour of formic aldehyde a material degree of penetration ensues.

In testing for formaldehyde in meat, 10 grammes of minced

meat may be heated for five minutes (on a boiling-water bath) with water to every 10 c.c. of which have been added 2 c.c. of a 1 per cent. solution of phenylhydrazine hydrochloride. After heating, the liquid is cooled and filtered from the coagulum through a loose plug of cotton-wool. To 12 c.c. of the filtrate are added 1 c.c. of 5 per cent. potassium ferricyanide solution and 4 c.c. of concentrated hydrochloric acid for each 12 c.c. of water and phenylhydrazine reagent employed in the test. By comparison of the colour with standards made from standard formaldehyde solutions the amount of formaldehyde in any given meat sample may be estimated.

Salicylic Acid and Salicylates.—If milk is to be tested, take 50 c.c. and dilute it with 50 c.c. of water, then add 5 drops of acetic acid and 5 drops of a solution of oxide of mercury in nitric acid, and well shake. After the albumin is coagulated, the mixture is filtered. The clear filtrate is then shaken with 50 c.c. of equal parts of ether and light petroleum, and the ether is subsequently allowed to separate out. Draw off the ether, place it in a clean vessel, and evaporate to dryness. The residue dissolved in a few drops of hot water and tested with 2 drops of a 1 per cent. solution of ferric chloride gives a violet or purple colour in the presence of salicylic acid.

The amount may be very approximately determined by matching the colour produced with a standard solution of salicylic acid (0·05 per cent. salicylic acid in 50 per cent. alcohol) to which 2 drops of the iron solution are added. In the quantitative estimation S. Harvey recommends the employment of an iron-alum solution (1 per cent.), instead of ferric chloride, as the colour struck is purer, deeper, and more permanent; and Allan advises that definite amounts of salicylic acid should be added to a liquid of the same kind as that in which the acid is to be determined, so that the error of experiment may be ascertained. Distilled water must be used in all the dilutions, as the salts of the alkaline earths affect the violet tint.

To test for salicylic acid in beer or wine, the liquid should be acidulated with sulphuric acid and well shaken with an equal amount of a mixture of ether and petroleum naphtha; let stand, and then pipette off the ethereal layer and evaporate down to a few c.c.; add a little water and a few drops of dilute ferric chloride solution and filter, when the filtrate, in the presence of salicylic acid, will be of a violet-purple colour. Or the liquid may be

distilled, when the acid will chiefly be found in the last fraction of the distillate, which should be tested with the ferric chloride.

A trace of salicylic acid (like citric acid) appears to be occasionally a natural constituent of genuine wine, and it has therefore been proposed that no more than 2 ounces of wine should be taken for the purpose of testing for the addition of the preservative. The trace naturally present in wines appears to be about 0·001 gramme per litre.

Benzoic Acid and Benzoates.—C. Revis recommends the following procedure for testing for the presence of benzoic acid in milk, and the writer finds it very satisfactory:

One hundred c.c. (not less) of milk are diluted with an equal volume of water, and, after the addition of 5 c.c. of 10 per cent. sodium carbonate solution, heated in boiling water for two to three minutes; 10 c.c. of 20 per cent. calcium chloride solution are then added, and the heating continued, until coagulation of the casein, etc., is complete. The liquid is then cooled and filtered, and the filtrate neutralized to litmus-paper with hydrochloric acid. Ten c.c. of Fehling copper sulphate solution (not mixed with the tartrate solution), followed by 10 c.c. of a solution of potassium hydrate (containing 31·18 grammes per litre), are now added, and the liquid again filtered. The filtrate is poured into a separating funnel, acidified with hydrochloric acid, and extracted with about 50 c.c. of ether. The ether is then washed three times with a little distilled water. About 10 c.c. of water are now added to the ether in the funnel, together with 1 drop of phenolphthalein solution, and then a saturated solution of barium hydrate added gradually, until, on violent shaking, the aqueous layer remains pink; this is then filtered off into a porcelain basin and evaporated to about 5 c.c. The contents of the basin are filtered into a test-tube, and dilute (1 in 100) acetic acid dropped in until the pink colour is discharged, after which 2 more drops are added. The liquid is then tested with 1 drop of 10 per cent. neutral, freshly prepared solution of ferric chloride, when, in the presence of benzoic acid, a fine reddish-yellow precipitate forms. This method will detect 0·02 per cent. of benzoic acid. When testing cream, 50 c.c. are diluted to 200 c.c. with distilled water, and the mixture is treated as directed above.

Saccharin.—One hundred c.c. of the material is acidified with dilute sulphuric acid (1 in 4), and extracted with a mixture of equal parts of ether and light petroleum. The ethereal extract

is then drawn off and evaporated at a gentle heat, when the residue will have a sweet taste if saccharin is present. Two c.c. of a saturated solution of sodic hydrate are then added, and heat applied until the residue dries and the mass fuses. The heat is maintained for half an hour, when the saccharin is converted into salicylic acid. The residue is acidulated with dilute sulphuric acid, and the ferric chloride test applied. If salicylic acid is originally present, it may be removed by dissolving the residue from the ether extract in 50 c.c. of dilute hydrochloric acid, adding bromine water to excess, well shaking, and filtering.

Should the ether extract contain benzoic acid in addition to saccharin, the extract may be heated at 110° to 115° C. until the whole of the benzoic acid has sublimed, the saccharin remaining unchanged.

Sulphurous Acid and Sulphites.—If beer or wine is mixed with hydrochloric acid, and granulated zinc is added to the small flask containing the liquid, a lead acetate paper placed over the mouth of the flask becomes discoloured ($SO_2 + 3H_2 = SH_2 + 2H_2O$).

For a quantitative estimation, add 5 c.c. of phosphoric acid to 200 c.c. of wine or beer, and distil 100 c.c. in a current of CO_2, receiving the distillate into 20 c.c. of decinormal iodine solution. After the distillate has been collected, the iodine solution must still be coloured (*i.e.*, must contain some free iodine), or the experiment must be repeated with more of the iodine solution.

The sulphurous acid liberated by the phosphoric acid is oxidized to sulphuric acid ($SO_2 + 2H_2O + I_2 = H_2SO_4 + 2HI$), and the sulphuric acid in the distillate may be precipitated by HCl and $BaCl_2$ as $BaSO_4$. One milligramme of $BaSO_4 = 0{\cdot}274$ milligramme SO_2.

Or the following simple method may be employed: 25 c.c. of normal potash solution are added to 50 c.c. of the sample, and the mixture is shaken occasionally for fifteen minutes. Ten c.c. of 25 per cent. sulphuric acid and a little starch solution are then added, and the mixture is rapidly titrated with decinormal iodine solution until a blue tint remains for a few minutes. Each c.c. of iodine solution required $\times 0{\cdot}00127 = SO_2$ in grammes per 100 c.c.

Sulphites in meat may be tested for by taking 100 grammes of the finely ground meat and making a thin suspension with water; acidify with phosphoric acid; then distil, and collect distillate in a few c.c. of distilled water, to which add a few drops of

bromine water and boil; then add a little barium chloride solution, when a white precipitate will indicate SO_2.

The amount of sulphites recoverable on analysis falls far short of that originally present.

Fluorides are used as preservatives for sweet wines and beer, and more rarely for milk, butter, and meat. A little ammonium carbonate is added to 100 grammes of the material so as to render it slightly alkaline; it is then brought to the boiling-point. A few c.c. of calcium chloride solution are added, and the boiling continued for ten minutes. The precipitate is collected, washed, dried, and ignited. When cold, a few drops of strong sulphuric acid are added, and the dish is covered with a piece of glass partly covered on its under surface with paraffin. The dish is then heated over warm water for one hour, when the glass will be etched if fluorides are present. Small quantities can only be discovered by working on large amounts of the material.

Hydrogen Peroxide.—This powerful oxidizing agent has been suggested by Budde as a harmless means of preserving milk and cream. To 10 c.c. of milk sample add 1 c.c. of 1 per cent. ortol solution (freshly made), when, in presence of hydroxyl, a dull crimson colour is produced, unless the milk has been heated above 72° C., when the addition of a little fresh milk is required, because the reaction is dependent on peroxydase, and this dies out in stale milk and is destroyed by heating. Paraphenylenediamine similarly gives a blue coloration. In the presence of organic matter hydroxyl splits up slowly into water and free oxygen, and so, after six to eight hours, may not be found.

Schryver suggests a physico-chemical method for estimating antiseptic values. The principle of the method consists in the determination of the inhibition of the rate of putrefaction of a given mixture from the presence of varying quantities of antiseptic, as indicated by the rate of chemical change of this mixture. For this purpose a solution of 5 per cent. gelatine and 1 per cent. peptone infected by fæces may be employed. As putrefactive bacteria break down the gelatine, an alteration in the physical properties of the mixture leads to an altered degree of electrical conductivity, which may be measured.

The Colouring Agents Employed in Food.

The question of the detection and identification of artificial colouring matters in articles of food is of considerable importance to the food analyst in view of the ever-increasing use of the aniline and other synthetic dyes, some at least of which cannot but be regarded as objectionable.

Harmful Colouring Agents.—Those formerly employed were mostly of mineral origin, but at the present day the sulphate of copper affords practically the sole important instance of the use of mineral agents in the coloration of food (*vide* pp. 362, 363).

Gamboge and *picric acid* are said to be occasionally used for the production of a yellow colour.

Gamboge is very insoluble in water, but readily so in alcohol. Its presence is detected by dissolving out the colouring matter from the article by alcohol, filtering, and then, when water is added, the gamboge resin is precipitated; this precipitate will dissolve in ammonia with the production of a blood-red colour.

Picric acid (tri-nitro-phenol) can be extracted by alcohol and ether; it is detected by drying on the water-bath after the addition of a solution of the cyanide of potassium and caustic potash, when the blood-red colour of the iso-purupraté of potassium forms.

Certain *aniline* colours are liable to contain arsenic, as, for example, in the production of rose aniline arsenic acid is used as an oxidizing agent, and crude oil of vitriol may have been employed in the manufacture of other dyes; but if tested and found to be free from this metal, they are mostly harmless. Some, however, such as naphthol green, metanil yellow, Victoria yellow, Martius' yellow, Bismarck brown, methylene blue, and gentian violet, are more or less poisonous, apart from the presence of arsenic. As a rule, only minute quantities of these aniline colours are employed at a time.

To distinguish between Victoria yellow, picric acid, and Martius' yellow, evaporate off the spirit by which the colour was extracted, and cautiously taste for the bitter flavour of picric acid; then treat with a little dilute hydrochloric acid, and when the solution has become nearly decolorized, introduce a piece of zinc for about an hour. Picric acid yields a fine blue, and Victoria yellow a blood red. Martius' yellow gives a golden-

yellow solution from which acids separate a white precipitate; this is not the case with picric acid.

Aniline colours may be detected by the iso-nitril test: To a little of the extract an equal amount of potash-lye is added, followed by 2 drops of chloroform; if the whole is gently warmed for a minute, and then boiled, the characteristic disagreeable odour of iso-nitril generally becomes perceptible. The well-known bleaching solution reaction for aniline can be readily obtained by adding an excess of the reagent to a solution of the aniline in water.

Aniline colours are largely employed. They are more readily soluble, cheaper (in consideration of the amount employed), and they withstand the effect of light and time better than the ordinary vegetable colours available for colouring food.

The various **harmless colouring agents** are very numerous, and are mostly of animal and vegetable origin. They have almost completely replaced the harmful colouring agents formerly used. To enumerate those which are most commonly employed in the production of various tints:

A *red, pink, crimson,* or *lake colour* is generally produced by either *cochineal* or the *aniline reds* (fuchsin and magenta).

The former colouring agent is derived from the cochineal insect, and is, like magenta, commonly employed. It can be detected by its character of turning violet with alkalies and yellowish-red with acids.

The reddish colours extracted from the root of the *madder,* from *beetroot,* and from *safflower,* are also used.

The colour due to safflower turns light brown and bleaches when treated with concentrated sulphuric acid.

Logwood is also of common employ, and may be demonstrated by extracting with alcohol and then adding alum and ammonium carbonate, when a lavender colour results.

A *yellow, amber,* or *orange hue* is commonly imparted by *annatto, turmeric, aniline orange, marigold* (the extract of which yields a permanent dark olive-green with concentrated sulphuric acid), *chrysophanic acid* (which is extracted from rhubarb, and yields a fine purple colour with caustic potash), and *saffron.*

Annatto (which is obtained from the seed of a plant named *Bixa orellana*) is very extensively used as a colouring agent, and, like turmeric and saffron, it is readily soluble in alcohol, though not in water. If in either case the alcohol is subsequently

evaporated down and the residue touched with a drop of concentrated sulphuric acid, annatto and saffron turn dark blue, changing to green, which in the case of saffron turns to a reddish-brown; and saffron furnishes a very peculiar odour. Turmeric yields a violet-red, and will turn brown with alkalies.

A *violet* or *blue colour* is frequently derived from the use of the *aniline* blues and violets.

Methylene blue may be detected by adding hydrochloric acid to the extract, when a greenish precipitate results. Zinc dust reduces it, but the colour returns after exposure to the air.

Methyl violet extracted and treated with hydrochloric acid yields first a green and then a yellow colour.

Indigo is extensively employed, and it sublimes in dense violet vapours when the article is burned. The colour is discharged by the permanganate of potassium in the presence of potash solution.

The blue colour of *litmus* affords another means of such coloration. This substance is derived from *Rocella tinctoria* and from certain other lichens.

A *purple colour* is usually formed from a mixture of blue with some vegetable pink, such as rose-pink, logwood, and cochineal.

The various *shades of brown* are most commonly imparted by heating *sugar* to various stages ("caramel"); and a *green colour* is now almost invariably obtained by the use of the *chlorophyll* extracted from plants rich in this substance, such as parsley, spinach, etc. The *aniline* greens are also used.

If the substance is thoroughly macerated in a small quantity of slightly warmed distilled water and the colouring matter is dissolved out by these means, and the coloured solution when treated with a solution of sodium hypochlorite and gently warmed becomes decolourised, then it is probably an aniline colour. Most harmless colouring agents are soluble in alcohol, ether, or chloroform. Further, the ash of the substance will furnish no inorganic constituents that are capable of forming the colour present. In this connection it should be noted that metallic mordants have been used for fixing organic colours.

If the colour is insoluble in water and not bleached by sodium hypochlorite, it is in all probability of mineral origin, and the presence of copper, zinc, lead, chromium, and arsenic should at least be tested for. Such tests, in many cases, are best made from a solution of the ash of the substance.

Almost all the azo-dyes, now so largely in use, are decolorized by stannous chloride in an aqueous solution containing hydrochloric acid.

Arata's wool-test is valuable. White wool is cleansed by boiling for a few minutes with caustic soda, and then thoroughly washing with water to remove all alkali. About 100 c.c. of the liquid or extract is mixed with 1 per cent. of potassium acid sulphate, and raised to the boiling-point. The wool is then soaked in the liquid for several minutes, removed, well washed in boiling water, and dried. Many of the artificial colours dye the wool, and the dye is either not changed by ammonia solution, or, if changed, is restored by further washing with water. The natural colouring matter of wines, fruits, cane-sugar etc., is not taken up by the wool, and any slight colour which may be taken up is changed by ammonia, and is not restored by washing.

Meats (especially sausage meats) may be coloured, and coal-tar colours are mostly employed. Water or alcohol will extract sufficient for the wool-test.

A simple test for added colouring matter in milk is to let it stand. In the case of genuine, high-coloured milk most of the colour rises with the cream, but the bulk of any artificial colouring matter generally remains in the milk. A simple test for coal-tar dyes of the azo group is the addition of hydrochloric acid to the *fresh* milk, when a pink colour forms in their presence.

The use of any dye, harmless or otherwise, to colour food in order to conceal damage or inferiority should be prohibited. Certainly it should be illegal to colour milk.

Dirt in Food.—This may be defined as adventitious matter foreign to the special article of food under consideration, and derived in various ways during collection or manufacture, while exposed for sale, during transit to the retailer and consumer, and while in the hands of the consumer.

The microscopic characters of the dirt which gains access to milk has already been indicated, and such an examination of other articles of food often discloses the presence of clay and sand, soot, straw, hair, wool and cotton fibres, sawdust, vegetable matter, and motile organisms belonging to the infusoria. Bacteriologically the liquefying bacilli largely predominate.

As would be expected, the amount of dirt found varies greatly even in different samples of the same material. In such articles

as watercress, parsley, apples, tomatoes, cherries and grapes, currants and dates (exposed on stalls), sugar and salt, black pepper, the writer and Dr. Dove found dirt varying from $\frac{1}{2}$ grain to over 300 grains per pound.

Dirt containing micro-organisms, moulds, yeast, etc., may do considerable harm to the food itself, setting up fermentation, destroying its keeping powers, and thus making it unmarketable or unwholesome. Furthermore, the knowledge that food has been placed under uncleanly conditions is unappetizing, and there is always the danger involved in such dirty handling and storing that it may get contaminated by germs of communicable disease. Although this danger is not great in most articles of food, it is considerable in such an article as milk.

CHAPTER XV

ARSENIC IN FOOD—ARSENIC AND OTHER POISONOUS METALS IN WALL-PAPERS, ETC.—RAG FLOCK

It will frequently happen that the metal under examination is distributed in solid organic matter; in these cases it is necessary to destroy the organic matter before proceeding to test for metallic impurities.

There are several alternative methods of separating organic matter from inorganic:

1. The organic matter may be destroyed—

   (*a*) By careful ignition. But by this means some of the inorganic matter is apt to be lost. In addition to the losses indicated on p. 2—*i.e.*, when the solid residue of a water is ignited—arsenic and antimony may escape, and copper, mercury, and zinc may suffer partial volatilization. (The addition of concentrated sulphuric acid before ignition would, by forming copper and zinc sulphates, prevent any volatilization of these metals.)

   (*b*) By heating with oxidizing agents, such as a mixture of chlorate of potassium and hydrochloric acid.

   (*c*) The organic matter may be destroyed by Kjeldahl's process.

2. The colouring or organic matter may sometimes be separated by filtration or dialysis.

ARSENIC IN FOOD.

Of substances which are subject to risk of such contamination the majority are those in which glucose is used, or which (like glucose) are prepared by the use of a relatively large

amount of sulphuric or hydrochloric acid, which so generally contains arsenic.

Traces of arsenic have been found in jams, sweets, lemonade, liqueurs, sugar, glycerine, and treacle—all now largely manufactured from glucose—and also in sulphate of sodium, phosphate of sodium, carbonate of sodium and potassium, caustic soda, sulphurous acid, sulphites, borax, oxide of iron (used for colouring confectionery), etc. It has also been pointed out that the coke used for kilning barley in the preparation of beer may give off traces of arsenic when burned.

Traces of arsenic may gain admission to food even in the process of cooking, from the enamelled, etc., cooking utensils employed.

Traces are also to be found in the ash of many of the natural foods, and notably such vegetables as cabbages, turnips,

FIG. 81.—CRYSTALS OF ARSENIOUS ACID.

potatoes, spinach, haricots, peas, and lentils. It is not surprising, therefore, that Gautier and Bernand find that arsenic exists normally in men and animals.

The Royal Commission on Arsenical Poisoning suggested that action should be taken under the Sale of Food and Drugs Acts when any liquid is found to contain $\frac{1}{100}$ of a grain or more of arsenic in the gallon; or when any solid contains $\frac{1}{100}$ grain of arsenic or more in the pound.

The method (**Reinsch**) of examining beer for arsenic, which was recommended in 1900 by a strong committee of chemical experts, is as follows:

Two hundred c.c. of the beer are heated to boiling in a porcelain dish, 30 c.c. of pure hydrochloric acid are added, and then a piece of clean, bright copper-foil, $\frac{1}{4}$ inch by $\frac{1}{2}$ inch in size; the boiling is continued for forty-five minutes, the water lost by evaporation being replaced. If after that time the copper remains bright, no arsenic is present. If a deposit has been formed, the foil is washed successively with water, alcohol, and ether, dried at 100° C., and heated in a 2-inch reduction-tube—the upper part of which should be previously warmed. If any sublimate is obtained, it must be examined under a magnifying

power of 200 diameters, when, if arsenical, octahedral and tetrahedral crystals will be seen. Perfect octahedra are comparatively rare, the majority of the crystals being modified forms of the octahedron or tetrahedron. In the case of very faint sublimates the crystals have, as a rule, the appearance of minute triangles.

Sulphites of various kinds are often added to beer in small quantity as preservatives, and when present they cause the formation of black copper sulphide. This stain, however, remains unchanged when the copper is heated in the small tube,

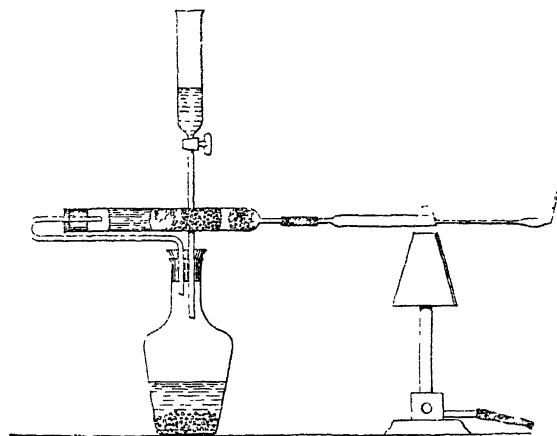



FIG 82.—MARSH'S APPARATUS FOR TESTING FOR ARSENIC.

and, of course, it furnishes no sublimate. Occasionally a slight film of organic matter diminishes the brightness of the copper, but this again could not possibly be mistaken for arsenic.

A. H. Allen advises that the operator should, as a preliminary treatment to eliminate sulphites, add hydrochloric acid and a little bromine water, and boil the liquid for a few minutes. To obviate the difficulty caused by the fact that arsenic acid only responds to Reinsch's test after prolonged boiling, and in presence of much acid, he adds a little solution of cuprous chloride in hydrochloric acid, which reduces the arsenic to the arsenious condition.

A joint committee of the Society of Chemical Industry and of the Society of Public Analysts reported (1901) in favour of **Marsh's process.**

In the horizontal drying-tube of the apparatus shown in

Fig. 82 a roll of blotting-paper soaked in lead acetate solution and dried is first placed, then a wad of cotton-wool, then granulated calcium chloride, and finally a thick wad of cotton-wool.

Mode of Testing Recommended.—The HCl employed must always first be freed from the usual traces of arsenic. To effect this it is well to add bromine and an excess of sulphurous acid to it, and to distil off and reject about one-fifth, the remainder of the distillate being generally quite free from arsenic, even if crude yellow hydrochloric acid has been employed. About 20 grammes of zinc are placed in the bottle, after washing with water to remove particles of dust which may contain arsenic; all parts of the apparatus are connected, and a sufficient quantity of hydrochloric acid allowed to flow from the funnel, so as to cause a fairly brisk evolution of hydrogen. When the hydrogen flame burns with a round (not pointed) tip, all air has been removed from the apparatus. The Bunsen burner should then be placed under the hard glass tube as shown in the figure, and more acid (10 to 20 c.c. is generally enough) run in. With pure materials no trace of a mirror is obtained in the contracted part of the tube just beyond the flame in half an hour. Great care must be taken that when additions of acid are made to the zinc no bubble of air is introduced, since in the presence of air the arsenic mirror may become black and unevenly distributed, instead of brown.

Should the blank experiment not be satisfactory, it must be ascertained by changing the materials methodically whether the fault lies with the acid, the zinc, or the apparatus.

If no trace of a mirror is obtained, 10 c.c. of the liquid to be tested and about 10 c.c. of hydrochloric acid are put into the funnel, and slowly introduced into the bottle without air-bubbles. Some materials (beers, for example) are apt to froth, hence the necessity for slow introduction. If after about ten minutes no mirror appears, another 10 c.c. of the liquid, with 10 c.c. of hydrochloric acid, are added, and the experiment continued for fifteen to twenty minutes, acid being from time to time added as may appear necessary.

Preparation of Standard Mirrors.—After a satisfactory blank experiment, a series of standard mirrors may be prepared under the following conditions:

A hydrochloric acid solution of arsenious oxide, containing in each c.c. 0·001 milligramme As_2O_3, is prepared by diluting a stronger solution with distilled water. Two c.c. of this solution

(equal to 0·002 milligramme of arsenious oxide) are introduced into the apparatus, a new tube having been joined to the drying-tube. If the zinc is sensitive, a distinct brown mirror is obtained after twenty minutes. It is important to note that some "pure" zinc is not, from a cause at present unknown, sufficiently sensitive;

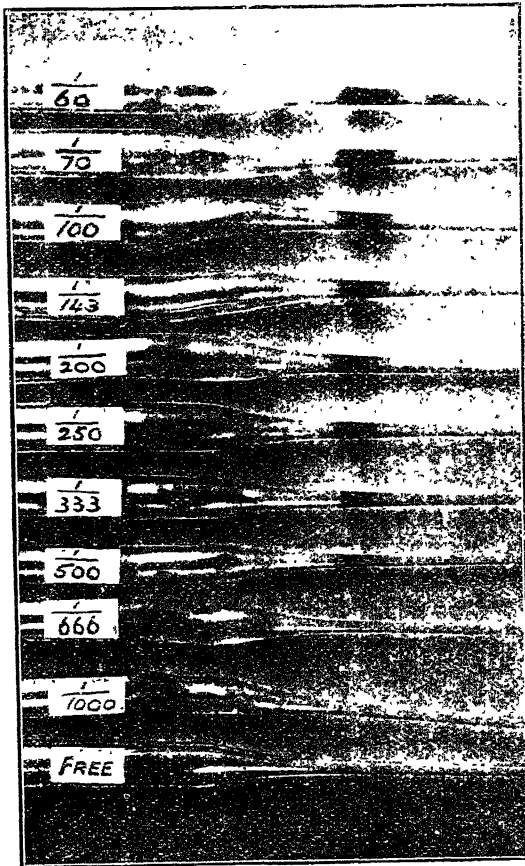



FIG. 83.—ARSENICAL MIRRORS (W. THOMSON).

that is to say, the addition of minute quantities of arsenic produces no mirror. The portion of the tube containing the mirror should be sealed off while still filled with hydrogen, as in contact with air the mirrors gradually fade. Mirrors are now similarly made with 0·004, 0·006, 0·008, and 0·01 milligramme of arsenious oxide. With a little practice it is easy to obtain the deposits of

arsenic neatly and equally distributed. The standard mirrors, properly marked, are mounted on a white card or porcelain slip. It is to be understood that the first stage of the collection of every mirror must be a blank of at least twenty minutes.

As an additional precaution a fresh tube should always be substituted for that containing the mirror, and the experiment continued for a further period of fifteen minutes. Should a second mirror be formed, the quantity of arsenic to which it corresponds is added to that shown by the first.

To obtain uniformly brown deposits of arsenic, the current of hydrogen should be slow, combined with a sparing evolution of arsenic hydride, the latter condition being brought about by the gradual addition of the arsenical substance to the Marsh apparatus. The rate of the hydrogen current may be judged by the size of the flame at the open end of the tube (W. Ackroyd).

W. Thomson has pointed out that there is an advantage in cooling the portion of the tube arranged for receiving the mirror; and also in always destroying (by oxidation) the organic matter, when this is markedly present, before commencing tests for arsenic.

It must be recognized that the tests are only approximate, and that mirrors corresponding to less than 0·003 milligramme of arsenious oxide cannot be safely relied upon. When a mirror has been obtained, a duplicate test should always be made in order to preclude error by accidental contamination.

The proof that a mirror is arsenical may be obtained as follows:

The narrow portion of the tube containing the mirror is cut off, the hydrogen replaced by air, and the ends sealed up. The tube, held in the tongs, is then drawn repeatedly through the flame of a Bunsen lamp until the mirror has disappeared. On cooling, minute crystals of arsenious oxide deposit, the sparkling of which can be seen with the naked eye if the tube be held before a luminous flame, and they can be readily identified under the microscope by their crystalline forms.

In order to obtain crystals easily examined under the microscope, Delépine's plan is to cause the crystals to form on a cover-glass. For this purpose he uses the contrivance shown and described in Fig. 84.

Gutzeit's test for arsenic is as follows: To a small flask containing pure Zn and dilute HCl, a drop of $PtCl_4$ and the concentrated liquid (beer, etc.) are added, and the hydrogen generated

is passed through a dry tube containing a dry strip of lead acetate paper; this strip of paper traps the sulphur compounds, and the black stain may be seen half-way up the paper; the

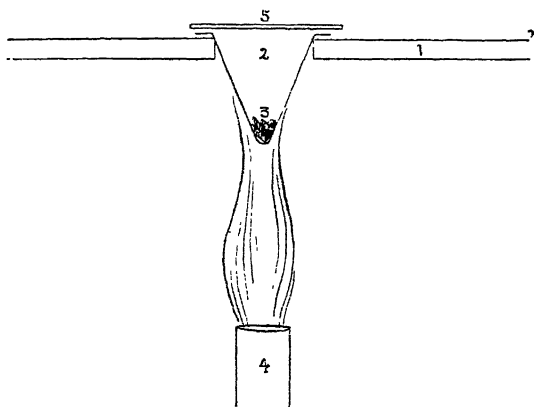



FIG 84.—S. DELÉPINE'S APPARATUS FOR THE COLLECTION ON A COVER-GLASS OF ARSENIOUS ACID FROM DEPOSITS OF ARSENIC ON COPPER.

1, Thick iron plate, which must not be allowed to get very hot during the heating of 3; 2 cone made of "electric" copper foil absolutely free from arsenic; 3, small pieces of copper covered with deposit of arsenic and placed in the portion of the cone which must be brought to a dull-red heat by means of the flame from a Bunsen burner (4); 5, cover-glass.

arseniuretted hydrogen escapes, and if a strip of dry $HgCl_2$ paper is held at the mouth of the tube a yellow stain is found on the paper. This reaction is exceedingly delicate.

THE EXAMINATION OF WALL-PAPERS, CURTAINS, CARPETS, LINOLEUM, ARTIFICIAL FLOWERS, ETC., FOR ARSENIC.

The dangers which may arise from the presence of this poisonous agent in wall-papers, etc., are well appreciated and have been frequently demonstrated. There is a widespread impression among the general public that green is the only colour likely to contain this dangerous agent; this is far from the truth, though the metal has been found more commonly in that colour than in any other.

Almost any colour may be arsenical; and it may be accepted that since the colour *green* is so generally associated with arsenic in the popular mind, that colour is the most likely to be kept

free from arsenic by manufacturers. Indeed, it is a fact that Scheele's green is never now employed for colouring wall-papers or carpets and curtains.

Arsenical compounds are sometimes also used as mordants to fix the dye upon materials.

The metal is commonly dissociated from arsenical wall-paper in the form of volatile arsenical compounds. Small grains of arsenious acid sometimes also enter the atmosphere, from carpets, rugs, and furs, in a suspended form, and (rarely) even metallic arsenic may thus be given off; so that a microscopic examination of the dust of a room may possibly disclose the presence of minute octahedral crystals and flakes.

Long ago Gmelin showed that of rooms with arsenical wall-paper, those situated on ground-floors or in other positions favouring dampness were most dangerous to live in. Abel showed that this was due to the action of moulds, which derived their necessary moisture and nourishment from the paste on the wall-paper. The nature of the arsenical compound given off is not known, but it possesses the garlic smell characteristic of arseniuretted hydrogen. *Aspergillus glaucus*, *A. niger*, and a form of *Mucor mucedo* can produce this change, but *Penicillium brevicaule* is the best organism for the following test: The suspected article, reduced to a state of fine division, is added to moistened breadcrumbs contained in a 100 c.c. flask. After sterilization the contents are inoculated with the mould, the cotton plug of the flask is covered by a caoutchouc cap, and the whole set to incubate for one or two days at 37° C. If arsenic be present, the garlic odour is readily detected on removing the caoutchouc cap. The method is even valuable when the arsenic is present in very small quantity.

If Scheele's green is suspected of furnishing the colour, a little of the paper or cloth may be thoroughly well soaked in ammonia, when a blue colour is created. In no case, however, can the employment of either Reinsch's or Marsh's test be dispensed with.

In applying Marsh's test, the paper, carpet, or cloth, etc., is cut up into small pieces, and these are introduced into the apparatus. Advantage should be taken of the pattern. When, for instance, this consists of flowers and leaves of different colours, these should be cut out, sorted according to their colours, and scrapings or extracts of each colour should be separately introduced into the flask and tested.

T. E. Thorpe recommends the following procedure: A weighed portion of the sample, cut into pieces of convenient size, is placed in a platinum dish of about 7·5 centimetres in diameter, and moistened with hot water. When the water has been absorbed by the fabric, 20 c.c. of arsenic-free lime-water and 0·5 gramme of calcined magnesia are added, the latter being stirred with a glass rod among the pieces of the fabric. The platinum dish is then placed on a hot plate or over a small Bunsen flame and the liquid evaporated. The dried material is then thoroughly charred and heated in a muffle furnace until practically all the carbon is burnt off. When cold, the ash is moistened with water and 20 c.c. of dilute sulphuric acid added. The dish is warmed, and the contents transferred to a flask of about 120 c.c. capacity. Half a gramme of potassium metabisulphite is added, and the solution boiled until free from sulphurous acid. The liquid is cooled, and diluted to a bulk of 50 c.c. in a calibrated flask or measuring tube. An aliquot portion may then be taken for Marsh's test.

The amounts of lime-water and magnesia given above have been proved by direct experiment to retain amounts of arsenic ranging from 0·0025 to 5 milligrammes when contained in 2 grammes of wool or paper.

The reduction of the solution with sulphurous acid before addition to the apparatus is necessary, since, under the conditions of the experiment, arsenic in the form of an arsenate or arsenic acid does not yield arseniuretted hydrogen.

Arsenic may exist in varying quantity in different materials—*i.e.*, from a fraction of a grain (of arsenious acid) to many grains per square yard; but since very small amounts will condemn the article containing it, there is often no necessity to go into the matter of a quantitative analysis.

The presence of arsenic in aniline dyes is rarely exemplified by the appearance of a rash upon the skin of those whose under-clothing is coloured by arsenical dyes. More especially is this the case with scarlet and blue stockings; and the legs in consequence are the commonest seats of such eruptions.

In order to test the extent to which arsenical colours are now employed, Dr. Dove undertook an inquiry, at the writer's suggestion, in 1913.

Of the forty-three materials examined, thirty-four (79·0 per cent.) gave negative results, two (4·6 per cent.) gave mirrors

corresponding to the presence of 0·005 milligramme of arsenic (As_2O_3) in the gramme of material (viz., 5 parts per million), three (6·9 per cent.) gave about half that quantity, and four (9·3 per cent.) only a very slight trace. Of the two articles which gave 0·005 milligramme of arsenic, one was orange colour, and the other yellow, and both were cloth materials; of the three which showed half the above amount, two were dark and light olive green cloths, and one a sort of mulberry-coloured silk; of the four showing only a very slight trace of arsenic, one was orange silk, and three were heliotrope, lavender, and emerald green cotton material. The cheap socks, which were green, blue, and violet in colour, gave negative results.

Eighteen wall-papers of different qualities, colours, and prices were next examined. When different colours were present, or even different shades of the same colour, each colour was separately tested. Ten (55·5 per cent.) of these materials gave negative results, two (11·1 per cent.) gave about 0·0025 milligramme of arsenic (As_2O_3), and six (33·3 per cent.) furnished but a very slight trace. The two which gave about 0·0025 milligramme of arsenic were a green paper with mauve flowers and a gamboge yellow paper with a flower pattern in a lighter shade. In the case of the first paper mentioned the arsenic was found to be almost wholly in the mauve flowers.

Zinc, chromium, and **tin** are other poisonous metals which have been found in coloured textile fabrics.

The presence of **lead** in carpets, curtains, etc., may possibly induce symptoms of chronic lead-poisoning; and large quantities are commonly contained in wall paints and papers, and in floor-cloths—chiefly red and white. An examination, therefore, for the presence and amount of this metal may sometimes become necessary.

By the Regulations of the Local Government Board, 1912, made under the **Rag Flock** Act, 1911, flock manufactured from rags, and used for the purpose of making any article of upholstery, cushions, or bedding, shall be deemed to conform to the required standard of cleanliness when the amount of soluble chlorine (in the form of chlorides) removed by thorough washing with distilled water, at a temperature not exceeding 25° C., from not less than 40 grammes of a well-mixed sample of the flock, does not exceed 30 parts of chlorine in 100,000 parts of the flock.

Example.—Forty grammes of the flock were placed in a large beaker, distilled water was added until a layer floated above the flock, the whole was then covered over and left until the following day. Clean muslin was then placed over a large filter-funnel, delivering into a 500 c.c. flask, and the soaked flock was emptied on to the muslin and well squeezed. It was next opened out, the water from the beaker poured on to it, and squeezed out, and finally a little distilled water was washed through the flock, until after squeezing it 500 c.c. of liquid had collected in the flask. After mixing thoroughly two quantities of 100 c.c., each were filtered through filter-paper. In one portion the chlorine was estimated by silver nitrate standard solution as described in water analysis, but as the fluid was highly coloured and contained sufficient organic matter to reduce some of the silver, the result was only taken as approximate.

The second portion of 100 c.c. was heated on the water-bath with plenty of strong nitric acid (to destroy organic matter), silver nitrate solution was then added in excess, and the mixture heated until the chloride of silver was all precipitated. The precipitate was next collected on a weighed filter-paper, the filtrate was again treated with nitrate of silver and refiltered, the second filtrate being quite clear, the precipitate was then washed, dried, weighed, and calculated to chlorine in parts per 100,000.

Supposing that the 100 c.c. of extract contains 0·026 gramme AgCl. Then the whole 500 c.c. contains 0·13 gramme AgCl.

But $Cl = \frac{35 \cdot 46}{143 \cdot 34}$ of AgCl.

$\therefore$ 0·13 gramme AgCl = 0·03 gramme Cl.

$\therefore$ there is 0·03 gramme Cl in 40 grammes of flock, or 75 parts per 100,000.

PART VI

THE EXAMINATION OF DISINFECTANTS

In judging of the value of a disinfectant the following points should demand consideration:

Its germicidal power, in presence of a small amount of organic matter; as to whether it is homogeneous and capable of remaining homogeneous under the conditions of use; at the strength in which it is employed in practice, whether it is poisonous to higher animals (including man), affects the skin, or injures textile articles and metal surfaces; whether it possesses deodorant properties; and its relative cost compared to other disinfectants.

Tar preparations contain: (1) Neutral bodies; light and heavy oils, with poor disinfecting properties. (2) Basic bodies; aniline, pyridine, etc., with marked disinfecting properties. (3) Phenols; soluble in alkalies, with considerable disinfecting properties (especially cresylic acid and the still higher homologues of carbolic acid).

The phenols are obtained from the distillates of coal tar. The fraction distilling from tar between 170° to 230° C. is called "carbolic oil," and consists chiefly of carbolic acid and naphthalene, but it also contains cresols and some higher homologues of phenol. The phenols are extracted from the mixture by means of caustic soda, the separated alkali solution is decomposed with acid, and crude carbolic acid separates as oil. Crude carbolic acid contains, besides carbolic acid, cresols and higher phenols. Pure carbolic acid can be separated by distillation. From 180° to 182° C. carbolic acid distils over. The tar acids boiling between 190° and 200° C. are chiefly cresols, and the various fractions boiling at higher temperatures contain other higher homologues of phenol.

A miscible carbolic disinfecting fluid may consist almost entirely of liquid tar-oils to which an alkali and a soap, resin, or gelatine have been added in order to emulsify; but some contain high percentages of phenols.

In making the dilutions of coal-tar disinfectants with hard water, some emulsions partly separate out almost at once; especially is this so in the higher strengths of 5 per cent. and over. This circumstance is explained by the fact that in coal-tar disinfectants the oils are generally combined either with soap or albuminous material, and large quantities of calcium and magnesium salts in water may cause a precipitation of the soap in a soap emulsion, whereas they have relatively little effect upon an albuminous emulsion. The writer and M. Wynter Blyth have pointed out the close relationship which exists between the germicidal values of the coal-tar disinfectants and the fineness of the emulsion, and that it is not possible by shaking up a disinfectant which has been de-emulsified by admixture with hard waters to restore the emulsion, or any but a small proportion of the loss of germicidal value.

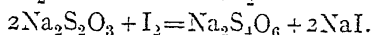
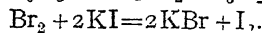
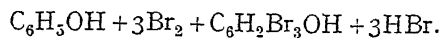
A good test for the presence of phenol, and one which serves to exclude creosote, consists in placing a few drops of spirit of nitrous ether in a test-tube, and then adding about 2 c.c. of a very dilute solution of the phenol; if sulphuric acid is now poured down the side of the test-tube a purple or pink colour forms, especially after standing awhile. No such reaction is obtained from a dilute solution of creosote (Eykman).

As a test for the presence of carbolic acid or phenol in a clear and colourless solution, ferric chloride solution may be added, when a purple colour indicates the presence of phenol or cresol. A better test for phenol is the addition of bromine water, which produces a white precipitate (tribromophenol). Both tests are rendered more delicate when applied to the first portion of the distillate of a suspected fluid.

A test for phenol in disinfecting powders may be performed by adding some of the powder to water, making acid with sulphuric acid, distilling over, and adding ferric chloride to the distillate.

Quantitatively, carbolic acid is estimated by the process of Koppeschaar. The phenol is precipitated as tribromophenol by the addition of excess of bromine solution. The surplus of bromine is determined by adding potassium iodide from which

the bromine displaces iodine, and the amount of the latter is found by titration with $\frac{N}{10}$ sodium thiosulphate solution.

$$C_6H_5OH + 3Br_2 + C_6H_2Br_3OH + 3HBr.$$
$$Br_2 + 2KI = 2KBr + I_2.$$
$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI.$$

The bromine solution used may be $\frac{N}{10}$, and should have its strength compared with the $Na_2S_2O_3$. The best working details of the process (L. V. Redman, A. J. Weith, and F. P. Brock) are as follows:

The *special solutions* required are:

1. A $\frac{N}{10}$ solution of sodium thiosulphate (24·8 grammes to the litre).

2. A $\frac{N}{10}$ bromide-bromate solution (2·76 gramme of potassium bromate and 15 grammes of potassium bromide per litre).

Solution 2 must be compared with solution 1 by adding acid and iodide of potassium (20 per cent.), and titrating the iodine liberated.

The process.—Into a 500 c.c. stoppered bottle put 50 c.c. of water and 5 c.c. HCl (S.G. 1·2).

Add 15 c.c. of the phenol solution, which has been diluted, if necessary, to approximately decinormal strength.

Slowly add, while shaking, enough $\frac{N}{10}$ bromide-bromate solution to furnish a permanent slight yellow. (The temperature of the liquid should be about 22° C., as low temperatures retard the rapid formation of tribromophenol.)

Restopper firmly and shake for one minute. Remove stopper, add 0·5 c.c. of iodide of potassium solution (20 per cent.), restopper, and again shake for one minute.

Next wash down the stopper and the sides of the bottle, and titrate with $\frac{N}{10}$ sodium thiosulphate, using starch (0·5 per cent. solution) as indicator, when the solution becomes almost colourless.

The difference between the amount of thiosulphate used and the known amount of bromide-bromate solution added represents the amount of the latter used up in the formation of tribromophenol. Each c.c. of the $\frac{N}{10}$ bromide-bromate solution =0·0015675 gramme of phenol.

The following procedure (advocated by the *Lancet*) for estimating the *tar acids* in disinfecting fluids is simple and serviceable: Ten grammes of the disinfectant are made up to 100 c.c. with

water, and thoroughly mixed; 100 c.c. of a saturated solution of baryta, to which a few crystals of barium oxide hydrate are added, are brought to the boil, and then rapidly filtered into a flask of about 300 c.c. capacity. The diluted disinfectant is slowly added to the hot baryta water, vigorously shaking the mixture all the time. This results in the separation of the soaps and resins. It is now filtered, and the filtrate made up to 300 c.c. with water, after washing the residue on the filter-paper. Half the filtrate —*i.e.*, 150 c.c.—is put into a separating flask, made acid with HCl (this liberates the phenols), and then 50 c.c. of ether added, and the whole well shaken. On standing, the ether, which has dissolved the phenols, will separate out. The brown solution below should be tapped off into another separating flask, and the ether into a weighed platinum dish. Another 50 c.c. of ether are added to the solution tapped off into the second separating flask, and the extraction repeated, and the ether is then added to the former ether. The entire ether is now driven off, the dish placed in a hot-air oven for ten minutes at a temperature of 50° C., and then reweighed. The weight of phenoloids so obtained multiplied by 20 gives the percentage of phenols present in the disinfectant. The phenoloids are now dissolved in caustic soda solution, and then made up to the 100 c.c. with water. Take 5 c.c. of this, dilute with water, make strongly acid with HCl, and run in a $\frac{N}{5}$ solution of bromine in caustic soda until a permanent yellow colour is produced. The number of cubic centimetres required, multiplied by 1·248, gives the bromine value, in terms of pure carbolic acid, of the percentage of phenols present. If this result agrees with the percentage of phenols by weight, the phenoloids are pure carbolic acid. If there is a great difference between the two results, carbolic acid may be considered absent, the disinfecting agent in that case being one or more of the homologues of carbolic acid.

For the determination of the *tar-oils in crude carbolic acid* the following simple method given by A. H. Allen will suffice: Introduce 10 c.c. of the sample into a graduated tube, and add gradually, noting the effect produced, four times its volume of a 10 per cent. solution of caustic soda, free from alumina. Then close the tube and agitate well. The coal-tar acids will be completely dissolved by the alkaline liquid; whilst, on standing, the neutral oils will form a separate stratum above or below the other, according as the admixture consisted of the light or heavy

"oil of tar." By the volume occupied by the oily stratum the extent of the adulteration is at once indicated. After noticing whether the tar-oils are light or heavy, a volume of petroleum spirit, equal to that of the sample, may be advantageously added; its employment facilitates the separation of the oily stratum, and renders the reading of its volume more easy and accurate. Of course, the volume of the petroleum spirit used must be deducted from that of the total oily layer.

The amount of *water present with phenol* may be found by shaking the sample in a graduated cylinder with half its volume of a saturated solution of sodium chloride. The reduction in the volume of the phenol indicates the amount of water present. If the sample is pure, it contains no water, but crude acids may contain 15 per cent. or over.

The specific gravity of crude carbolic acid should be between 1·050 and 1·065, and if it is below 1·050 it is probably adulterated with light tar-oil.

In some cases the base of *carbolic powders* is slaked lime, when the carbolate of lime formed is of little value as a disinfectant. When specifying for the supply of carbolic powder, a percentage of tar acids (generally 15 per cent.) should be demanded, together with the guarantee of an inert base, which does not enter into chemical combination. The carbolic acid is often added to silicious matter ("carbolized silicate powders") or to peat ("carbolized peat powders").

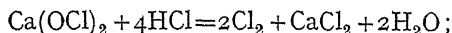
The percentage of *phenols and cresols* present *in carbolic powders*, in which phenols are not in chemical combination, may be readily arrived at by the following means: If the powder contains no lime, 50 grammes of the powder are thoroughly shaken up in ether, and thus all the free and available tar acids are abstracted; to this extract is added 50 c.c. of 10 per cent. caustic potash solution, and gentle heat is applied to drive off the ether. The alkaline liquid having been boiled down and then emptied into a graduated cylinder, 50 per cent. sulphuric acid is added to slight acidity; when cold, the tar acids separate out, and their volume can be read off; this volume × 2 will, of course, give the percentage amount. Then the percentage of phenols in the powder is to the amount read as 10·5 is to 10, for the specific gravity of phenol is about 1·050. For the extraction of the total carbolic acid in powders containing lime, in which the phenols are chemically combined, 50 grammes of the powder

are cautiously and slowly treated with sufficient 50 per cent. sulphuric acid in a mortar until a minute particle of the powder moistened with water gives an acid reaction when placed on litmus-paper. Calcium sulphate is thus formed, and the carbolic acid is set free. The powder may then be exhausted with ether, and the ethereal extract may be filtered into a flask containing 50 c.c. of 10 per cent. caustic potash solution. The contents of the flask are then well shaken up, and the ether driven off. The liquid is then emptied into a graduated cylinder, and 50 per cent. sulphuric acid is added to slight acidity, when the coal-tar acids that separate out completely on the liquid becoming quite cold $\times$ 2 will approximately represent their percentage volume.

The crude carbolic acid obtained should always be examined for neutral tar-oils, and the test described on p. 402 will generally be sufficient for all practical purposes.

Good carbolic acid powders generally contain from 12 to 18 per cent. of crude carbolic acid, but they are liable to lose 1 or 2 per cent. by volatilization. Half of the total oils in some powders consist of neutral tar-oils (Allen).

Bleaching Powder.—When treated with HCl, bleaching powder gives off chlorine:

$$Ca(OCl)_2 + 4HCl = 2Cl_2 + CaCl_2 + 2H_2O;$$

and the liberated chlorine will, by liberating iodine, turn a white potassium iodide and starch-paper blue (due to iodide of starch). To confirm the presence of bleaching powder, calcium should also be tested for.

To estimate the **available chlorine in chloride of lime, chlorinated soda, etc.,** decinormal solutions of iodine (12·69 grammes to the litre) and of arsenious acid (4·95 grammes of pure arsenious oxide and 20 grammes of sodium bicarbonate to the litre) are employed.

One gramme of chloride of lime is rubbed up with water in a mortar, and made up to 100 c.c. Ten c.c. of the turbid liquid (previously well shaken) are placed in a white porcelain dish, which thus contains 0·1 gramme of the chloride of lime. The arsenious solution is added to slight excess, as shown by the fact that iodide of potassium and starch-paper is no longer blued. Now add fresh starch solution, and run in the iodine solution until a faint blue tint remains permanent, when the amount of the iodine solution required corresponds to the excess of arsenious solution used. Deduct this from the number of c.c. of the arsenious solution added (for the two decinormal solutions are

chemically equivalent), and the difference represents the amount of arsenious solution which is oxidized by 0·1 gramme of chloride of lime. Each c.c. of the arsenious solution=0·00354 gramme of available chlorine.

In chloride of lime solution the available chlorine is that existing as hypochlorite, $Ca(ClO)_2$, which readily breaks up into $CaCl_2$ and 2O. When the arsenious solution is added it combines with the oxygen to form arsenic acid, and the starch is not permanently blued until all the arsenious acid is oxidized to arsenic acid; when added in excess some arsenious acid remains, and this is measured by the iodine.

Several estimations should be made and the mean figure taken, and the strength of the iodine solution should be checked against the arsenious acid prior to use.

Example.—Ten c.c. of the turbid solution containing 0·1 gramme of chloride of lime were taken; 9·3 c.c. of $\frac{N}{10}$ arsenious solution were added. Added $\frac{N}{10}$ iodine until blue tint remained; 0·3 c.c. were required. Therefore, available chlorine in the 10 c.c. of sample=9·3 – 0·3=9 c.c. $\frac{N}{10}$ arsenious solution. But 1 c.c. of $\frac{N}{10}$ arsenious solution=0·00354 gramme of chlorine.

∴ 9 c.c.=0·03186 gramme of chlorine in 10 c.c. of sample, or 0·1 gramme of chloride of lime.
=31·86 grammes of chlorine in 100 grammes of sample.

∴ There is approximately 31·86 per cent. of available chlorine present in the bleaching powder.

Formalin may be detected by distilling some of the liquid, adding to the distillate a drop of 5 per cent. carbolic acid, and running down the side of the test-tube strong sulphuric acid, when a crimson zone results if formalin is present.

A solution of the **perchloride of mercury** furnishes a yellow precipitate, soluble in excess, with potassium iodide.

Copper sulphate, zinc chloride, and ferrous sulphate in solution may be tested by methods previously indicated in dealing with water analysis.

A **sulphurous acid** solution will furnish a white precipitate with silver nitrate, which is soluble in nitric acid; or if some granulated zinc and hydrochloric acid are added to the solution, and a piece of moistened lead paper is held over the mouth of the flask containing it, the paper will be darkened from the production of sulphuretted hydrogen.

The sulphurous acid contained, along with other disinfectants, in some disinfecting powders may be estimated by shaking up 1 gramme of the finely crushed powder in a large quantity of freshly distilled water, so as to make the solution very dilute; 50 c.c. of decinormal iodine are run in; the mixture is then made distinctly acid with dilute hydrochloric acid, and the excess of iodine is titrated with decinormal thiosulphate. Each c.c. of the iodine solution reduced by the powder=0·0032 gramme of SO_2.

Permanganate oxidizes oxalic acid, in the presence of sulphuric acid, to CO_2 and H_2O.

To determine the presence of permanganate it is only necessary to acidify with dilute sulphuric acid, add oxalic acid and warm, when the pink colour disappears.

To test the strength of a solution of **permanganate** a known volume of a decinormal solution of oxalic acid (6·3 grammes per litre) is placed in a beaker, and sulphuric acid is added until the solution is strongly acid; the diluted permanganate solution is then run in from a burette until the pink no longer disappears.

$$5H_2C_2O_4 + K_2Mn_2O_8 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 10CO_2 + 8H_2O;$$

and therefore 5 equivalents of oxalic acid require for oxidation 1 equivalent of permanganate (containing 5 atoms of available oxygen).

Example.—Fifteen c.c. of the liquid permanganate disinfectant were added to 10 c.c. $\frac{N}{10}$ oxalic acid solution before the crimson colour remained. Therefore 15 c.c. of the permanganate disinfectant=10 c.c. of $\frac{N}{10}$ oxalic acid solution—or, since decinormal solutions are chemically equivalent, 10 c.c. of $\frac{N}{10}$ permanganate solution.

Therefore, 15 c.c. of the permanganate disinfectant solution= 10 c.c. of a $\frac{N}{10}$ solution.

But 1 c.c. of $\frac{N}{10}$ potassium permanganate=0·00316 gramme, and 10 c.c.=0·0316 gramme.

∴ 15 c.c. of permanganate solution=0·0316 gramme potassium permanganate, and 100 c.c.=0·21 gramme, or approximately ·0·21 per cent.

The disinfecting power of a disinfectant can only be gauged by direct experiments upon micro-organisms.

THE DETERMINATION OF ANTISEPTIC AND GERMICIDAL POWER.

The three factors—strength of the solution, duration of action, and nature of the material acted upon—cannot be disassociated. If, for instance, we ascertain that a given strength of mercuric chloride will kill typhoid bacilli in broth culture in half an hour, we should still be ignorant of the strength which would be sufficient in the same time to render typhoid fæces harmless as a factor in the spread of enteric fever.

It is very difficult to define satisfactory standard conditions for testing purposes, because in practice disinfectants are employed as germicides under a variety of conditions.

Madsen and Nyman, and also H. Chick, have shown that when the disinfectant is present in considerable excess, the process of disinfection proceeds in accordance with a definite law, the number of living bacteria per unit volume progressively and regularly decreasing with increase of time in a logarithmic ratio.

Investigations may be required to—

1. Determine the restraining and germicidal power of different substances in solution.

2. Determine the germicidal power of substances when volatilized.

To Determine Lethal Power.—Two separate determinations may have to be made, one for the bacterium and one for the spore, if spores are produced.

In ascertaining lethal power it is very important to be certain that none of the germicide is carried over into the cultivation solution, as a very small amount may be sufficient to inhibit growth. In practice it is extremely difficult to get rid of all traces of antiseptic.

The "garnet method" of Krönig and Paul (1897) is a valuable one, but on the whole the "drop method" is the most convenient method for determining the germicidal action of any given substance for the ordinary bacteria.

In the *garnet method* garnets of similar size are selected, and after careful cleaning are dipped into a filtered watery emulsion of sporing anthrax or other bacillus selected. The emulsion is allowed to dry on them in a thin film. The loaded garnets are then immersed in the disinfectant solutions under investigation. After definite periods of time the garnets are taken out,

the disinfectant carried over removed by gentle washing, and (if necessary) washed in agents (such as ammonium sulphide, if mercuric chloride is used) to render inert any trace of disinfectant. The bacteria or spores are then separated from the garnets by shaking them in water. Definite amounts of the washings are then cultivated and the bacteria counted.

It is convenient to compare the germicidal power with that of some standard disinfectant under carefully standardized conditions. Rideal and Walker's method is the best yet devised for this purpose.

In the *Rideal-Walker method* a carefully standardized pure carbolic acid solution is used as a control, accurate dilutions in sterile distilled water being prepared. A twenty-four hours' broth culture (Lemco), grown at 37° C., of *B. typhosus* is the test organism. A reaction of +1·5 for 100 c.c. broth is recommended. The temperature of the room should be from 15° to 18° C. during the experiment.

To 5 c.c. of a particular dilution of the disinfectant in sterilized water 0·2 c.c. of the broth culture is added, and the mixture well shaken. Then subcultures are taken every two and a half minutes up to fifteen minutes. The subcultures are made in broth and incubated for at least forty-eight hours at 37° C. Those with a growth are then entered in the tables.

A number of different dilutions of the disinfectant under examination, and also one or more dilutions of the carbolic acid, are tested *at the same time*, and under precisely similar conditions of temperature, amount of disinfectant solution used, quantity of typhoid broth culture added, etc. The efficiency of the disinfectant is expressed in multiples of carbolic acid performing the same work—*i.e.*, a dilution of the disinfectant which does the same work as the standard carbolic acid dilution is obtained; the ratio obtained by dividing the former by the latter is called the "carbolic acid coefficient." The results are conveniently recorded in tables, of which the following is an illustration:

B. TYPHOSUS TWENTY-FOUR HOURS' BROTH CULTURE AT 37° C.
ROOM TEMPERATURE 15° TO 18° C.

Sample.	Dilution.	Time Culture exposed to Action of Disinfectant in Minutes.						Subculture.	
		2½	5	7½	10	12½	15	Period of Incubation.	Temperature.
								Hours	Centigrade
Disinfectant w.	1 : 700	×	×	..	..	..	..	48	37°
Disinfectant w	1 : 800	×	×	×	..	..	..	48	37°
Disinfectant w	1 : 900	×	×	×	×	×	..	48	37°
Carbolic acid	1 : 90	×	×	..	..	..	..	48	37°

Carbolic acid coefficient = [illegible] = 7·7.

A test-tube rack with holes for thirty test-tubes in two rows; small flasks and covered vessels (Fig. 85); a dropping pipette standardized to deliver 0·1 c.c. of broth culture per drop; an inoculating needle with a platinum wire providing a loop 3 millimetres internal diameter at its end, are required.

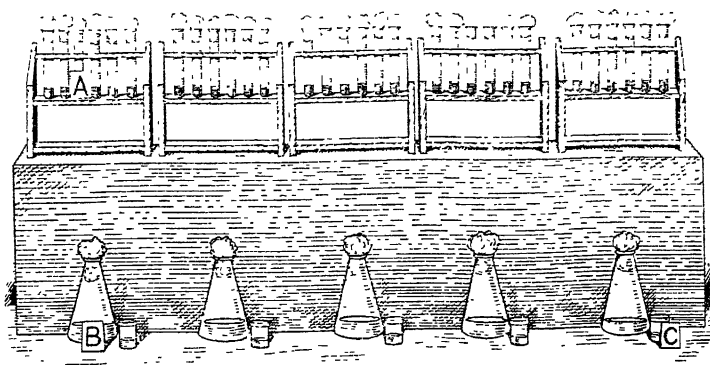



FIG. 85.—APPARATUS EMPLOYED IN THE RIDEAL-WALKER METHOD.
A, Test-tubes containing the nutrient medium to be inoculated. *B*, Flasks containing the different dilutions of phenol and the disinfectant under test. *C*, Small covered vessels containing 0·2 c c. of typhoid culture, and 5 c c. of particular dilutions of the disinfectant.

0·2 c.c. of the culture is placed in the five small tubes, and then 5 c.c. of the five different dilutions of the disinfectant are also added to the tubes at intervals of half a minute. When the fifth tube has been dealt with, the organisms in the first tube will have been exposed to the disinfectant for two minutes, so after waiting another half-minute, a loopful of the mixture in

the first tube is inoculated into the first broth-tube; and so at intervals of every thirty seconds loopfuls from the other four tubes are in turn inoculated into their properly labelled broth-tubes. When the fifth broth-tube is inoculated, the organisms in the first tube will have been exposed to the disinfectant for four and a half minutes, so the operator waits half a minute before again inoculating broth-tube No. 1; and so in seventeen minutes inoculations are obtained containing organisms which have been exposed to five different strengths of the disinfectants for six two and a half minute periods.

This method can be used to obtain the carbolic acid coefficients for other organisms—for example, *B. pestis, Sp. choleræa.*

Notes on the Method.—Use as stock organism *B. typhosus* from an agar slope culture that has been grown at 21° to 22° C. (70° to 72° F.) from two to five days, and removed by weekly transference for several uninterrupted generations from the original source (the human body). Carbolic acid is frequently contaminated with cresol, and cresol has approximately three times the bactericidal efficiency of phenol. In order, therefore, to secure uniform conditions of testing, it is necessary to work with carbolic acid of such purity that the solidifying point exceeds 40° with the thermometer in at least 50 c.c. of the liquid. A 5 per cent. by weight stock solution is then prepared, and standardized by titration with decinormal bromine. This keeps well, and is employed for making the necessary weaker dilutions for test purposes.

The composition of the broth employed is—

20 grammes of Lemco.
20 grammes of Witte's* peptone.
10 grammes of sodium chloride.
1 litre of distilled water.

This mixture is boiled for thirty minutes, filtered, and then neutralized with normal NaHO, using phenolphthalein as indicator. The broth is then made up to a litre, 15 c.c. of normal HCl is added, and the whole filtered and sterilized. The exact reaction of the broth in which the test organism is grown for the twenty-four hours prior to the test is a matter of considerable importance as affecting the coefficient obtained. The writer

* The peptone employed is an important matter. If this cannot be obtained, Allen and Hanbury's "Eupeptone" is suitable.

has known a faultily made broth to reduce coefficients very considerably.

The method has been subjected to considerable criticism, but it is much employed for standardizing disinfectants for manufacturers' purposes, and in the specifications of tenders for supplying disinfectants it is often required that disinfectants quoted should possess a certain carbolic acid coefficient as determined by the Rideal-Walker process.

To obtain identical results a number of variants have to be controlled, even such apparently trivial matters as the composition of the broth, its age, the particular strain of bacillus, and the variation in temperature of medication, exercise considerable influence upon the results. The resisting powers of various strains of the organism introduce a very great effect. The writer has obtained from the same disinfectant on the same day coefficients of 12 and 20 by employing respectively a fresh and resistant strain and an old attenuated one. It is not safe to accept as final the results of only one series of observations, and the mean of several coefficients is desirable.

In particular the method, and all similar methods, must not be taken as furnishing without much modification a guide to the *practical* use of disinfectants.

The practical utility of any disinfectant depends mainly upon how much it is influenced by the presence of organic matter. The efficiency of some disinfectants is greatly impaired by the presence of organic matter, while for others a less diminution of power is so caused. For example, Martin and Chick* showed that when a 3 per cent. suspension of dried finely-divided fæces is used, the efficiency of phenol is reduced by about 10 per cent., while that of the emulsified tar acids is reduced from one-third to one-eleventh of the primary value. The soluble commercial cresols occupy an intermediate position, the reduction depending upon the solubility. The reduction in the case of the emulsified tar acids is found to be higher the finer the emulsion. Some disinfectants are more efficient against one species of bacteria, others against another. In the case of spores metallic salts are most efficient. The removal of an emulsion of higher phenols by bacteria is in the first instance a process of adsorption; disinfectants which form fine emulsions possess superior efficiency, because, owing to this adsorption, the bacteria rapidly

* *Journal of Hygiene*, 1908, vol. viii., p. 654.

become surrounded by the disinfectant in much greater concentration than exists throughout the liquid.

As the Rideal-Walker method does not take into account the influence of the presence of organic matter, various attempts have been made to obviate this difficulty. For this purpose the addition of gelatine, serum, urine, milk, fæces, etc., has been suggested by different workers, so that the germicidal power of the disinfectant may be tested in the presence of organic matter. None of these additions are altogether satisfactory, and it cannot be said that a suitable method has yet been evolved. The effect of the addition of these organic substances is in every case to considerably lower the coefficient obtained with what may be styled the naked germs; but the coefficient of some disinfectants (for example, potassium permanganate) is lowered to a much greater degree than others.

The action of antiseptics upon certain special organisms cannot be tested by the above method. As a good illustration of this the determination of the germicidal action upon tubercle bacilli may be mentioned. The fresh sputum may be spread upon slips of wood or other substance, and dried in a desiccator over sulphuric acid. Only completely dried slips should be used. The slips are soaked in different strengths of the germicidal solution under examination for a definite time (*e.g.*, three hours). The slips are then washed in sterile water, and the dried expectoration scraped off, made into an emulsion with sterile water, and injected into a series of guinea-pigs. If tuberculosis develops it is obvious that all the tubercle bacilli were not killed. By using an appropriate series of dilutions the correct lethal strength for the tubercle bacillus, under the conditions of the experiment, can be ascertained.

If the test organism produces spores, it must be incubated first under optimum conditions for spore formation, and cultures used which contain large numbers of spores.

To Test the Action of Volatile Disinfectants.—Broth cultures of different organisms may be used. *B. typhosus*, *B. diphtheriæ*, and *B. anthracis* are convenient to employ.

Sterile strips of linen or wood may be soaked in these solutions, then removed and dried at 37° C. in a vacuum over sulphuric acid. Such inoculated strips are exposed to the action of the gaseous disinfectant, present in known percentage, for definite but varying periods.

Some of the strips should be exposed freely to the disinfectant, others should be placed in the centre of rolled blankets, etc.

After the required time, the strips are inoculated into sterile broth-tubes, which are incubated and examined for growth.

The dried strips are conveniently carried in sterile Petri-dishes.

All the different factors of time, percentage of disinfectant, temperature, and humidity of atmosphere, should be carefully noted.

In the case of most volatile disinfectants a sufficiency of moisture must be present in the air of the room if the gas is to exert its disinfecting properties under the most favourable conditions. Thus, sulphurous acid gas generated into an atmosphere saturated with moisture has almost double the disinfecting power of the gas generated in a dry atmosphere; and formic aldehyde vapour, being most efficacious at a temperature of 70° F. and a relative humidity of 70 per cent., is incapable of producing its best results if the temperature and humidity of the air of the room are much below these optimum conditions.